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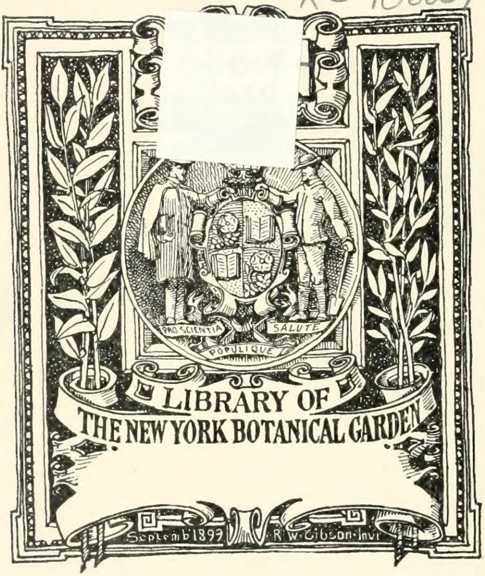
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ON THE MALE GAMETOPHYTE OF PICEA CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 200

(WITH PLATES XV XIX AND ONE FIGURE)

From
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ON THE MALE GAMETOPHYTE OF PICEA CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 200

A. H. HUTCHINSON

(WITH PLATES XV–XIX AND ONE FIGURE)

The male gametophyte of *Picea excelsa* has been described by STRASBURGER, MIYAKE, and POLLOCK. At the shedding stage, as recorded by STRASBURGER (1), there are two disintegrating prothallial cells, a stalk cell, a body cell, and a tube nucleus. MIYAKE (2) verified this account; also described the pollen tube stages and the division of the antheridial cell into stalk and body cells. POLLOCK (3) noted certain variations in the gametophyte at the time of pollination. This account deals with the early stages of development in the male gametophyte of *Picea canadensis*.

The staminate cones were collected from trees growing near Lake Simcoe, Ontario, Canada. Daily collections were made from May 2 until May 15, the time of shedding. The usual time for pollination in this locality is about two weeks later.

Nomenclature

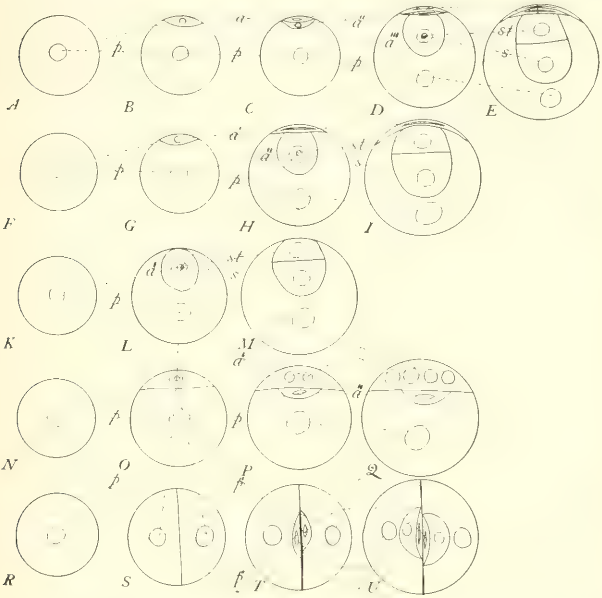
The nomenclature used in accounts of male gametophytes has varied according to the character used as a basis for the system, whether it be size, position, or the writer's conception of origin or function of the different cells. Early in the nineteenth century FITZSCHE described *Pinus* as having a large central vesicle and disintegrating bodies against the wall of the pollen grain (*Zwischenkorper*). MEYEN (in 1839) stated that the *Zwischenkorper* were cells, and that one of them served as a stalk of attachment. JURANYI (4) reported that in *Ceratozamia* the pollen mother cell divided into a large and a small daughter cell (*kleine Tochterzelle*); that the latter divided to form two, and that the inner of these gave rise by division to an inner cell and an end cell. These three cells were collectively known as the cell body (*Zellkörper*). Until 1891 the tube nucleus (*grosse Zelle* or *freigebildete Zelle*) was believed to be the fertilization nucleus. At that time BELAJEFF (**6**) showed that in *Taxus baccata* the larger cell is not the generative cell; but that the small cell divides in the tube and one of the derivatives becomes the generative cell.

In 1892 STRASBURGER (1) described *Ginkgo biloba* as having two prothallial cells, representing a vegetative prothallus, and an antheridial cell, which are successively cut off from the pollen cell; the antheridial cell divides into stalk and body; the latter produces the sperms. The large pollen nucleus, because of its foremost position in the tube, was called the tube nucleus. The general conception of origin and function is the same today. It is not surprising, however, that, with such a variety of types, different investigators have since used different terms to designate similar cells.

The system of nomenclature to be used in this account has been made necessary by the nature of the gametophytic development. The primary cell (*P*) is regarded as retaining its identity, just as an apical cell. In the pollen tube stages it is represented by the tube nucleus. The successive divisions of the primary nucleus are known as primary divisions; the cells cut off are called, tentatively, the first, second, or third primary derivatives (a', a'', a''' in figs.). Divisions of the latter cells are called secondary. The cell which later divides to form male nuclei is termed spermatogenous (*s* in figs.); the sister non-functioning cell is the sterile cell (*st* in figs.). The mother cell of a spermatogenous and a sterile cell is called an antheridial cell.

Development of the male gametophyte

The first primary division is variable; the cell wall may cut off a lenticular polar cell (fig. 7); it may be oriented at right angles to the longitudinal axis, cutting off approximately one-third of the protoplasmic mass (figs. 6, 33, 37, 53); it may be in the plane of the vertical axis, in which case two nearly equal cells result (figs. 2, 10, 11, 13, 31, 32); it is often inclined (figs. 28, 29); and occasionally no dividing wall is formed, the two resulting nuclei being then free in the cytoplasmic mass (figs. 8, 9, 12). The further development of the resulting cells is largely determined by the nature of the first primary division. When the primary derivative is small and lenticular, it rapidly degenerates (figs. A-I and 20–26 etc.); when the division is median or nearly so, each of the two cells formed has the power of repeated division, giving rise to a



FIGS. A–U.—A diagram to illustrate five types of development in male gametophytes of *Picea canadensis:* *P*, primary cell; *a'*, *a''*, *a'''*, the first, second, and third (potentially) antheridial cells; *s*, spermatogenous cell; *st*, sterile cell; the dotted lines indicate origin and sequence; relative size is also shown; figs. A–E, three primary divisions, first and second cells cut off evanescent, third by a secondary division produces a spermatogenous and a sterile cell; figs. F–I, two primary divisions, second cell cut off from primary cell functions as antheridial; figs. K–M, first primary derivative functions as antheridial cell; figs. N–Q, two primary divisions give rise to two antheridial cells, repeated divisions of the first produce four free nuclei; figs. R–U, primary cell divides to form sister primary cells, each of which produces an antheridial group, a bi-antheridial gametophyte.

so-called double gametophyte (figs. R–U and 28, 29, 31, 32); when the first primary derivative is surrounded by cytoplasm it divides, giving rise to a spermatogenous and a sterile cell (figs. K–M and 9, 12, 30). In this case there is no further division of the primary cell; the first primary derivative becomes the functioning antheridial cell. Again, if the first cell cut off contains sufficient protoplasm it may divide once or even twice to form as many as four free nuclei. When this occurs, the primary cell soon ceases to divide and begins to disintegrate (figs. N–Q and 28, 29, 33). The first primary derivative may function as an antheridial cell by directly dividing to form a spermatogenous and a sterile cell, by repeatedly dividing to form a number of free nuclei, or by becoming primary in nature and hence developing along with its sister cell to form a bi-antheridial gametophyte.

When the first primary cell is evanescent, a second primary division takes place. Nor is it uniform. Frequently the primary cell approaches the first primary wall before dividing, and it may come into contact with this wall. The second primary derivative is then cut off as a lenticular cell against the wall of the first and soon disintegrates (figs. C–E and 23, 25, 26). If, however, it remains imbedded in the cytoplasm of the primary cell, it divides to form a spermatogenous and a sterile cell (figs. F–I and 25, 38). Hence the second primary derivative may function as an antheridial cell.

When, as it has been hitherto described, the first and second primary derivatives are evanescent, a third primary division takes place, and the last cell cut off functions as the antheridial cell (figs. A–E and 42–51).

Since the spermatogenous cell may originate from the first, second, or third primary derivatives, we are forced to the conclusion that these cells are all potentially antheridial, one or in some cases two functioning as such. They may be known as evanescent or functioning antheridial cells, as the case may be.

Development; time; nutrition

Growth is exceedingly rapid; in three days the diameter of the pollen grain is doubled, its volume becoming four times as great. In *Pinus* "the mature pollen grain has the same size and form as the microspore just prior to germination" (**15**). When two evanescent cells are cut off, these divisions take place before the increase in size. They follow one another in rapid succession; all stages of the first two primary divisions are to be found in the same sporangium. A resting period, that is, a period during which mitosis ceases, but during which there is a great increase in size and apparently in food supply, precedes the formation of the functioning antheridial cell, whether it be the first, second, or third primary derivative (figs. D, H, L, O, S and 30, 38, 44, 45). Since this last primary division and the secondary division to form the spermatogenous cell and the sister sterile cell are to be found in the same sporangium, it is evident that they are closely consecutive. The complete development is extremely rapid; on May 3 only one-celled stages were to be found, while on May 6, or sooner, the pollination stage had been reached. Trees on a sunny hillside shed the pollen at once; others retained it for ten days without further development. The functioning antheridial cell is imbedded in the cytoplasm of the primary cell, as shown above, and an increase in the size of the pollen grain precedes mitosis of the former cell. Evidently nutrition is a factor in determining the fate of an antheridial cell; in other words, whether the first, second, or third shall function as such.

Degeneration

In lenticular cells which contain a minimum of cytoplasm surrounding the nucleus, the latter does not pass out of telophase (fig. 26); the chromosomes contract, become globular, and finally disintegrate as irregularly granular masses (figs. 20, 21, 43), or accumulate at the periphery of the nucleus, giving it a vaginated appearance (fig. 45). When these cells collapse, double darkly-stained bands appear in cross-section. In *Picea canadensis* the intine does not imbed these degenerating cells. The first primary wall elongates as the pollen grain increases in diameter (figs. 37, 38, 50, 51); often it has the appearance of a third wall (fig. 50) which is attached to the intine near the origin of the wings. The disintegrating cell contents remain within the original walls; the latter meanwhile become elongated and thickened.

Degeneration may occur in any part of the gametophyte. Frequently the second primary derivative degenerates before the first (fig. 37). If sister primary cells are formed, the struggle resulting from their parallel development is generally so great that disorganization of both results (fig. 32). Usually one gains the ascendency. Often, after as many as three cells have been formed, one of the antheridial groups is crowded against the wall; irregular cavities appear in the cytoplasm (fig. 31); the protoplasm contracts and accumulates in masses of globules; and the nuclei becomes massed or uniformly granular (figs. 33, 53). When an extreme development of the first antheridial cell occurs, the primary cell as well as the secondary antheridial cell may disintegrate (fig. 33).

Mitoses

There are two types of mitoses; that characteristic of primary divisions, and that of secondary divisions. The latter does not differ essentially from ordinary somatic mitoses; the former is quite distinctive in its characters.

The nuclei and chromosomes are decidedly kinetic. Just before mitosis, the primary nucleus moves to a more or less polar position. Frequently it comes in contact with one of the primary walls (figs. 14, 15, 16, 43). There is the usual movement of the chromosomes to form the central plate at metaphase, and the separation of chromosome groups during anaphase. After the two nuclei have been formed, the one which is polar retains its position, while the primary nucleus moves to its central or supracentral position (figs. 5–7; 19–25; 43–45).

The changes in the volume of the nuclear space are very marked. During prophase a slight expansion is followed by a contraction (figs. 14, 15, 16, 28) which continues until the disappearance of the nuclear membrane. The chromosomes at the poles during early telophase aggregate into compact masses (figs. 4, 18, 47, 49); the nuclear membrane is formed, and the nucleus expands until it becomes three or four times its original size. There is an associated accumulation or disappearance of food particles in the surrounding protoplasm. This may be regarded as evidence in favor of LAWSON'S (7) explanation of similar phenomena, namely, that they are due to osmosis. Moreover, the increase in size is most rapid when the nucleus is surrounded by most cytoplasm; the primary nucleus soon regains the size characteristic of the resting stage (figs. 6, 9, 18, 20, 21).

Chromatic structures

The resting nucleus contains several nucleoli. In early prophase "condensing bands" and "zig-zag threads" of chromatin become differentiated; definite looped chromosomes are formed (figs. 15, 35). Only in rare cases could their double nature be seen at this stage; it would seem that the halves remain rather closely appressed until metaphase. The nucleoli are present until late prophase (figs. 16, 35, 48). The chromosomes contract before aggregating in a definite cell plate (figs. 16, 48); during anaphase they are characteristically V-shaped (figs. 2, 3, 17). In early telophase compact chromatic aggregations are formed (figs. 4, 18, 41, 47, 49). These soon become irregularly vacuolate, and as the vacuoles increase in size, anastomosed bands of chromatin become differentiated (figs. 5, 22, 24, 43). The process is similar to that described by SHARP (8) in *Vicia*. The bands become more irregular in outline, and a number of nucleoli appear (figs. 23, 25, 44). As the number of nucleoli decreases, they become individually larger (figs. 20, 45, 6, 8, 46, 48, 50). The irregular bands are replaced by zig-zag threads and the nucleus passes again into the resting condition.

Achromatic structures

The achromatic structures in all primary divisions are most characteristic. The spindle fibers are inconspicuous during anaphase (figs. 2, 3, 36, 42); in many cases they can be distinguished only with difficulty (fig. 17) and are only slightly more definite than the vague radiations in the polar cytoplasm (figs. 3, 36). In early telophase there are very definite strands between the daughter nuclei. These are arranged in the form of a hollow cylinder (figs. 4, 18) which gradually broadens (figs. 19, 43) and moves toward the pole, partly enclosing the antheridial nucleus (figs. 5, 21, 24). Usually the fibers come in contact with the cell wall, the free ends swing outward, and so remain as curved or radiating strands (figs. 7, 23, 44, 45). The cell plate forms late; it is most definite after the fibers have taken their final position (figs. 6, 7, 8, 41, 45). These mitoses are similar to that described for *Abies balsamea* (**9**).

The cell plate is evidently associated with the formation of the cell wall. In fig. 45 the arched fibers remain only at one side, but here there is a distinct cell plate and the cell wall is curved outward to meet it. When the wall is formed the fibers disappear. In many cases no cell plate could be seen (figs. 5, 21, 22, 23), and the division in the cytoplasm is continuous with the end of the fibers (fig. 25).

There is abundant evidence that the achromatic fibers are definite structures which change their position. The fact that there are groups of spindle fibers which have no immediate connection with nuclear membranes or chromosomes is further evidence for their individuality (fig. 8).

Secondary divisions have markedly different characters. A polar cap is formed during prophase (fig. 48); the spindle fibers are more strongly developed during metaphase (fig. 38); the cell plate forms early (figs. 47, 49), and the spindle fibers retain their original positions. The similarity of secondary divisions in the first and third primary derivatives is illustrated by figs. 47 and 49.

Chromatic extrusions

In the early stages of the gametophyte, darkly staining bodies occur in the cytoplasm. When the primary nucleus is in the resting stage, these bodies appear as spherical masses surrounded by a clearer area (fig. 34); when the primary cell is in active mitosis these extrusions become fragmented (fig. 35). These bodies originate from wandering chromosomes which escape during mitosis. Separate chromosomes are found near the primary cell wall during metaphase (fig. 36). Evidently these never take part in cell plate formation. They contract into spherical masses and wander into the cytoplasm. In other cases, during late anaphase, several chromosomes prematurely contract to form a more or less compact mass, thereby separating themselves from the chromosomes which later undergo a similar change (fig. 3).

Discussion

1. *Double pollen grains*

There are accounts of double pollen grains occuring in a number of species. Probably first was CHAMBERLAIN'S (**10**) description of *Lilium tigrinum*. "In about 20 cases there was a distinct wall dividing the microspore into two nearly equal parts." Both cells contained starch. His fig. 20 shows one of the cells containing two nuclei "which seem to represent generative and tube nuclei." One of the cells was regarded as prothallial, the other as antheridial. SCHAFFNER (**11**) found compound grains where two or more of the spores of a tetrad clung together (*Typha latifolia*). GUIGNARD (**12**) and Miss PACE (**13**) figure four microspores of an orchid within a common wall dividing to form tube nuclei and generative cells. COKER (**13a**) describes double grains in *Larix europea*. His fig. 6 is similar to my fig. 13; his fig. 8 corresponds to my fig. 12. He suggests that "the mother cell had only divided once, so that only two instead of four pollen grains were formed." In some of these grains "division proceeded as usual except that only one prothallial cell is evident" (cf. fig. 32). POLLOCK (**3**) has described a number of variations in the pollen grain of *Picea excelsa*. "In the material examined, the proportion of double pollen grains was found to be 2.4 per cent in a count of 1120. The three or four cells lying along the dorsal side of the pollen grain of this type do not constitute a prothallium or gametophyte of unusual size. They constitute the smaller portion of a pollen grain separated by a division wall into two nearly equal portions, each of which may form a typical antheridium." Double pollen grains have been variously interpreted. In *Picea canadensis* a study of the stages of development has shown that the two cells from which the double grain arises are the result of a primary division of the microspore (figs. 11, 13), and that one of these cells corresponds in origin to the more usual evanescent cell. All gradations between an equal division and one which cuts off a lenticular evanescent cell have been found (figs. 6, 7, 10, 11, 13). In the double pollen grain of *Picea* one of the antheridial groups is homologous with the usual evanescent polar cell.

2. "*Prothallial cells*"

Several species have been described in which the "prothallial" cell has the power of division. Among the number are *Ginkgo biloba*, by STRASBURGER (**1**); *Picea excelsa*, by MIYAKE (**2**) and POLLOCK (**3**); *Abies balsamea* (**9**); *Agathis*, by JEFFREY and CHRYSLER (**14**); *Podocarpus*, by COKER (**13a**); and *Dacrydium* by Miss YOUNG (**16**). The similarity of the generative cell and the prothallial cells is pointed out by Miss YOUNG: "In *Dacrydium*, as in *Podocarpus* and *Abietineae*, a third cell is cut off from the main body of the spore. It overlies the others and is so similar to them that, but for its subsequent behavior, one might think it a prothallial cell. It is the generative cell, generative in the sense that it is the ancestor of the spermatogenous cell. This and the second prothallial cell now divide." The first prothallial cell may also divide. Again: "at shedding the pollen grain contains the body cell and five free nuclei. The nucleus of the body cell is indistinguishable from those of the prothallial cells and the tube nucleus." POLLOCK (**3**) states that in a large proportion of the gametophytes of *Picea excelsa* there is only one prothallial cell. BURLINGAME (**20**) says: "in *Podocarpus* one primary prothallial cell may be cut off, after which the free nucleus divides to form the free spermatogenous cell and the tube nucleus; or two primary cells may be cut off before the tube nucleus is separated from the primary spermatogenous cell." In these two species, as well as in *Picea canadensis*, the antheridial function is not limited to a definite primary derivative. It has been established that "prothallial cells" and generative cells may be similar in appearance; that frequently they are similar in their power of division; the similarity is further emphasized by the presence of "prothallial cells" in the pollen tube. The present account has emphasized the similarity in the origin, and has shown that potentially there is a similarity in function; that any one or sometimes two of the primary derivatives may be antheridial. To what extent we are justified in suggesting that these phenomena are indicative of a multi-antheridial ancestral form only further research can determine.

Relationships

No one character is sufficient to establish relationships of plant groups. Since similarity of male gametophytes gives only one-sided evidence for the relationship of species or genera, the discussion will be limited to comparison of types. In Taxodineae and Cupressineae the gametophyte consists of an antheridial cell, which may divide before or after shedding, and a tube nucleus; no evanescent cell is present. Similar gametophytes are found in *Picea canadensis* (fig. 30). In cycads two cells are cut off from the primary cell, one of which is antheridial (cf. fig. 38). The shedding stage characteristic of the abietinean gametophyte (also of *Ginkgo* and *Ephedra*) contains two more or less evanescent cells and an antheridial cell, which may or may not divide, beside the tube nucleus (cf. figs. 50, 51, 52). The podocarp type is similar, but the polar cells are not evanescent and frequently divide (cf. figs. 39, 40, 52). A massive polar tissue containing free nuclei, which LOPRIORE (18) regards as antheridial, is characteristic of the araucarian type. A similar gametophyte is shown in fig. 33. The male gametophyte of *Picea canadensis* is in a state of unstable equilibrium. Slight differences in conditions are sufficient to shift the balance in one of several possible directions. The resulting forms correspond to the various types of gametophytes found in gymnosperms.

Summary

In the male gametophyte of *Picea canadensis*, one, two, or three potentially antheridial cells are cut off from the primary cell; one of these divides to form a spermatogenous and a sterile cell; the others, when formed, are more or less evanescent. Occasionally there are two functioning antheridial cells, resulting in a bi-antheridial gametophyte.

The writer is indebted to Professor JOHN M. COULTER, Dr. CHARLES J. CHAMBERLAIN, and Dr. W. J. G. LAND for many suggestions and criticisms.

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EXPLANATION OF PLATES XV–XIX

The drawings (figs. 1–53) have been made with the aid of the Abbé camera lucida. The original magnification was 2000. A reduction of one-half has been made in reproduction.

FIGS. 1–13.—The first primary division; the primary cell retains its identity; the first primary wall cuts off an antheridial cell.

FIG. 1.—Primary cell.

FIG. 2.—A division at right angles to the longitudinal axis.

FIG. 3.—A division in the plane of the axis; a chromatic extrusion is being formed and is separating from the nuclear chromosomes.

FIGS. 4, 5.—Telophases: the primary nucleus and spindle fibers separating.

FIGS. 6, 7, 8, 11.—Telophases: each shows the cell plate in one of 4 positions; in fig. 8 both nuclei have escaped from the spindle fibers.

FIGS. 9, 12.—Two free nuclei in common cytoplasm; compare fig. 8.

FIGS. 10, 13.—The primary cell has given rise to two daughter cells, each of which may function as a sister primary cell.

FIGS. 14–27.—The second primary division.

FIG. 14.—The primary cell in contact with the first evanescent antheridial.

FIGS. 15, 16.—Prophases.

FIG. 17.—Anaphase: spindle fibers indistinct.

FIGS. 18–25.—Telophases: illustrate migration of primary nucleus and spindle fibers; fig. 24, a view from upper pole; figs. 23–25, the formation of the cell wall.

FIGS. 26, 27.—Pollen grains with primary cell and two non-functioning antheridial cells; compare size with fig. 1.

FIG. 28.—First antheridial ("prothallial") cell in division.

FIG. 29.—Primary cell; also first and second antheridial cells; the former has divided to form two nuclei.

FIG. 30.—First antheridial cell has divided to form a spermatogenous and sterile cell.

FIGS. 31, 32.—Two sister primary cells (cf. figs. 11 and 13) have given rise to a bi-antheridial gametophyte (disintegration).

FIG. 33.—Four free nuclei, products of first antheridial cell; the primary cell and second antheridial cell disintegrating.

FIGS. 34–36.—Chromatic extrusions.

FIG. 37—The position of the first primary wall and the large polar cavity is to be noted.

FIG. 38.—The second antheridial cell dividing.

FIGS. 39–40.—Laterally placed derivatives of first and second antheridial cells.

FIGS. 41–46.—The third primary division.

FIG. 41.—A polar view.

FIG. 46.—Large primary nucleus (tube nucleus): the third antheridial cell just before mitosis; the second in normal condition except for compression; the first disintegrated.

FIG. 49.—Mitosis in the first antheridial cell (cf. fig. 28).

FIGS. 47, 48, 50, 51.—Division of the third antheridial cell to form a spermatogenous and a sterile cell.

FIG. 52.—Showing polar cells, the products of secondary divisions.

FIG. 53.—Shows disintegrating derivatives of the first antheridial cell; a sterile cell; a spermatogenous cell and the primary nucleus (or tube nucleus).

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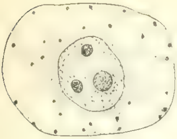

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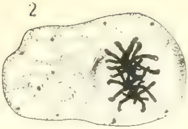

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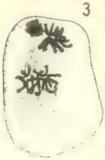

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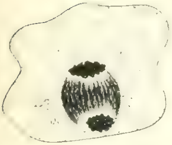

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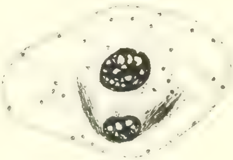

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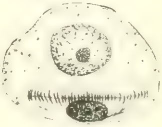

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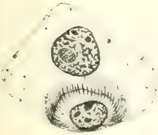

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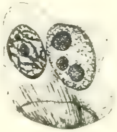

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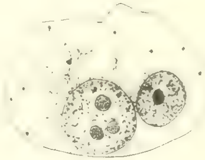

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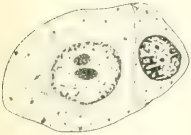

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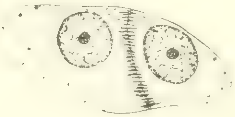

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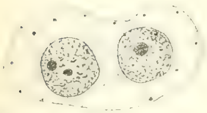

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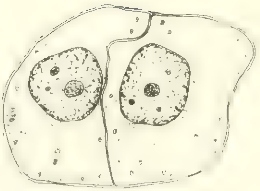

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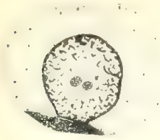
14



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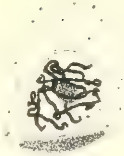

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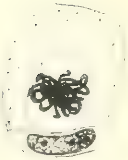

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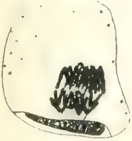

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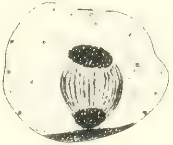

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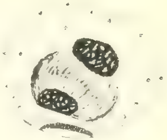

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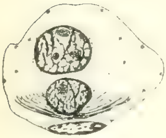

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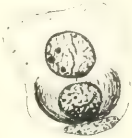

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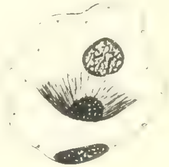

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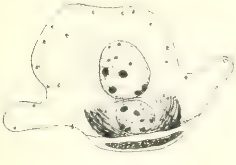

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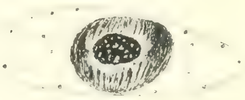

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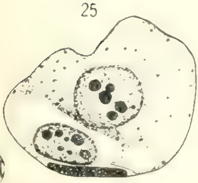

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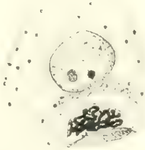

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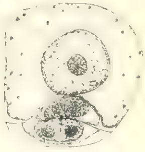

HUTCHINSON on PICEA

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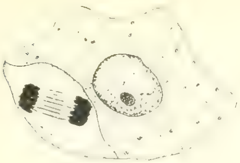

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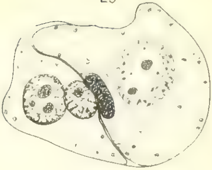

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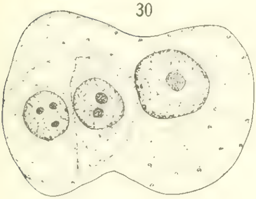

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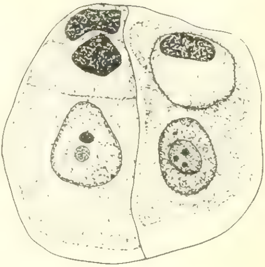

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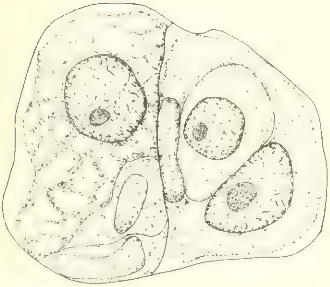

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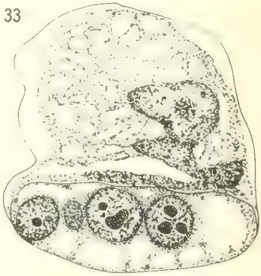

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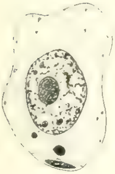

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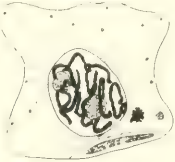

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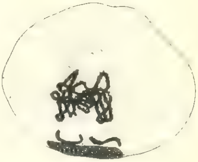

HUTCHINSON on PICEA

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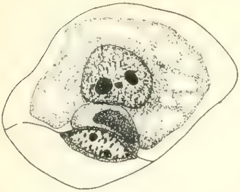

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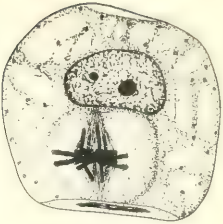

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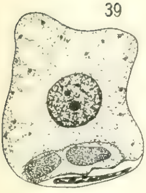

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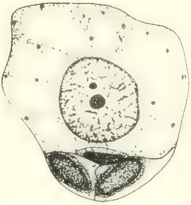

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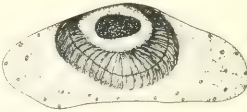

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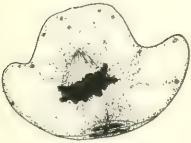

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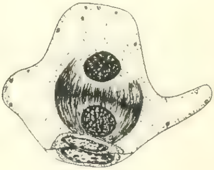

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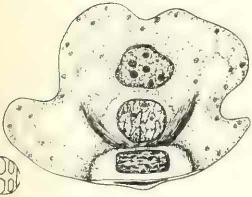

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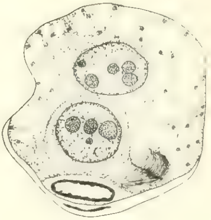

HUTCHINSON on PICEA

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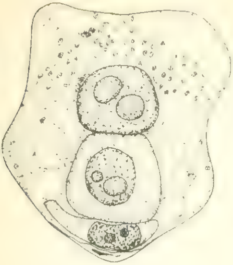

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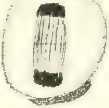

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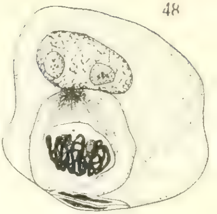

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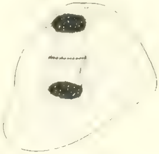

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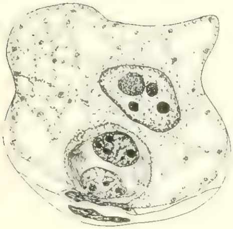

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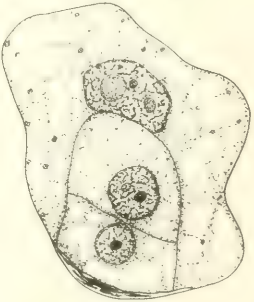

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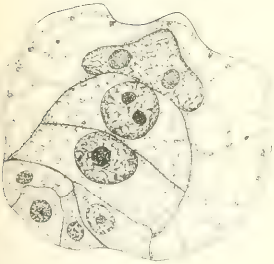

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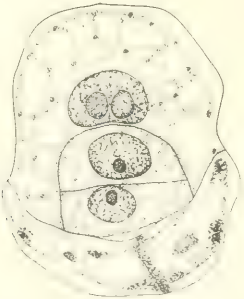

HUTCHINSON on PICEA

STUDIES IN THE GENUS BIDENS. II

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 201

(WITH THREE FIGURES)

EARL E. SHERFF

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STUDIES IN THE GENUS BIDENS. II

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 201

EARL E. SHERFF

(WITH THREE FIGURES)

Bidens acuticaulis, sp. nov.—Herba annua, tenerrima, 4–8 dm. alta; caule acute angulato, ramoso; ramis adscendentibus, acute angulatis et fere subalata, remote pubescentibus. Folia opposita, petiolata, petiolo adjecto 1–4 cm. longa, pinnata aut irregulariter bipinnata; foliolis (et lobis) linearibus, 0.5–1 mm. latis. Petioli 2–10 mm. longi. Capitula multa, terminalia, 3–4 mm. alta et 2.5–3.5 mm. lata (frutescentia demum 1–1.2 cm. alta et 4–5 mm. lata), ligulata. Involucrum basi pubescens; squamis duplici serie dispositis; exterioribus linearibus, plus minusve pubescentibus, 3–4 mm. longis; interioribus lanceolatis, marginibus diaphanis 1.5–3 mm. longis. Ligulae circiter 4, parvae, sub-flavidae aut fere albae, 2– (4–) striatae, 3–5 mm. longae. Paleae lineare-lanceolatae, striatae, marginibus diaphanis. Achaenia linearia, remotissime pubescentia, margine adscendente-ciliato, apice erecto-hispido et biaristato aristis retrorsum hamosis, 6–12 mm. longa.

John Gossweiler 4052, in herb-grown woods, Angola, April 4, 1906 (type in Herb. Brit. Mus.).

Bidens rufovenosa, sp. nov.—Herba erecta, perennis (?), 4–6 dm. alta; caule subtereto, striato, glabro, plus minusve ramoso; ramis (aut ramulis) monocephalicis. Folia opposita, petiolata, petiolo adjecto 4–6 cm. longa, pinnata, spinuloso-ciliata, supra sparsim et brevissime spinuloso-pubescentia, infra sparsim pubescentia et ad venas minute rufo-tomentosa;[1] foliolis lateralibus lanceolatis (aut foliorum superorum lineare-lanceolatis), incisis aut longe dentatis; foliis supremis integris et lanceolatis, aut ternatis, foliolis integris et lanceolatis; petiolis basi connatis, foliorum inferiorum angustis et 1–1.5 cm. longis, foliorum superorum brevioribus aut absentibus. Capitula ligulata, frutescentia 1–1.3 cm. lata et 0.8–1 cm. alta. Involucri squamae membranaceae, margine diaphanae, duplici serie dispositae; exterioribus (7–8) linearibus, 3–5 mm. longis; interioribus lanceolatis, dimidio longioribus. Ligulae (in specim. exsicc.) aurantiacae, anguste lanceolatae, apice integrae, ad basem minute hispidae, 7–9-lineatae, 1.5–1.8 cm. longae. Paleae membranaceae, lineares, margine diaphanae, 6–8 mm. longae. Achaenia linearia, subplana, una facie valde unicostata, adscendente-ciliatae, supra adscendente-hispidae, biaristatae aristis brevibus et nudis, 8–9 mm. longa.

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[1] Dr. S. ECKERSON, of the University of Chicago, has kindly made for me a critical examination of a leaf from the type, and she reports the color of the pubescence due to a red stain produced in the mucilage cells along the veins.

John Gossweiler 4176, among the ferruginous rocks near the fort at Kabango, Princip. Amelia, Africa, Dec. 1907 (type in Herb. Brit. Mus.).

Bidens cinerea, sp. nov.—Herba, annua (?), erecta aut adscendente-erecta, cinerea; caule striato, squarroso-hispido, subtetragono, monocephalico (aut ramis monocephalicis). Folia opposita, petiolata, tuberculato-scabrida et minute spinuloso-setosa, ternata aut pinnata, petiolo adjecto 2–6 cm. longa; lobis dentatis aut lobulatis, ovatis aut lanceolatis, brevioribus paulo aut etiam dimidio quam petiolis; petiolis spinuloso-setosis, 0.5–2 cm. longis. Capitula terminalia, ligulata, pedunculis 6–10 cm. longis, flosculis hermaphroditis exsertis. Involucrum hispidum, squamis duplici serie dispositis; exterioribus circa 8, linearibus, 4–5 mm. longis; interioribus dimidio longioribus, lanceolatis, margine diaphanis. Ligulae flavae, lineari-lanceolatae, apici integro, 7–9-striatae, circa 1.2 cm. longae. Paleae lineares, membranaceae, margine diaphanae, circa 8 mm. longae. Achaenia linearia, nigra, compressa, uno facie unicostato, striata, adscendente-hispida, biaristata, aristis retrorsum hamosis, circa 1.1 cm. longa.

Lieut. *C. S. Smith*, May, 1893, Kilimanjaro, Africa (type in Herb. Kew).

Bidens punctata, sp. nov.—Herba annua, erecta, gracilis, ± 5 dm. alta, caule subtetragono, glabro (aut nodis sparsissime longo-piloso), striato, supra plus minusve sparsim ramoso; ramis subtetragonis aut teretis, tenuibus, striatis, glabris (nisi nodis pilosis); ramulis monocephalicis. Folia opposita, tripartita (infima et suprema integra), reflexa luce nigro-punctata demum, ciliata, supra subglabra, infra pallidioria et sparse longo-hispida; lobis lateralibus brevibus, linearibus aut lanceolatis, integris (aut non saepe ad basim lobatis), basi saepe subangustatis; lobo terminali elongato, lineari aut lineari-lanceolato, integro (aut ad basim 1–2-dentato); petiolis plus minusve hispidis, basi connatis, 0.4–1 cm. longis. Capitula terminalia, longe (6–12 cm.) et tenuiter pedunculata, ligulata, capitula ligulata 2 cm. lata. Involucrum basi hispidum, squamis duplici serie dispositis; exterioribus (5–8) lineari-spathulatis, glabris aut hispido-ciliatis (aut etiam sparse hispidis), 2–4 mm. longis; interioribus lanceolatis, membranaceis, striatis, margine diaphanis, sparsim hispidis, 5–8 mm. longis. Ligulae flavae, lineari-lanceolatae, apice integro, 5–9-lineatae, 8–10 mm. longae. Paleae lineares, striatae, membranaceae, margine diaphanae, demum 6–7 mm. longae. Achaenia linearia, compressa, nigra, marginibus et faciebus tuberculatis aut tuberculato-hispidis, apici hispido pectinatim, biaristata, aristis 1–2 retrorsum hamosis.

Archdeacon *W. P. Johnson* 343, Tumbi (Makapula), April 27 (1901?; type in Herb. Kew); *idem* 341, Tumbi hill, Tumbi, April 27 (1901?).

The description here given is drawn mainly from the type sheet in Kew Herbarium, although certain dimensions, etc., are taken from *Johnson* 341 (also at Kew), a sheet with a larger and more matured plant. It may be remarked here that the punctate character noted above for the leaves is to be met with in *Bidens Taylori* and *B. Baumii* (see below), also a small number of other species.

Bidens vincaefolia Karsten et Schultz Bipontinus, sp. nov.—Herba, perennis (?), volubilis (?), caule angulato aut tetragono, ramoso; ramis glabris aut subglabris, tetragonis, striatis. Folia opposita, petiolata, petiolo adjecto 2–3 cm. longa, tripartita aut raro indivisa, subcoriacea aut membranacea, subtus pallidiora et ad venas minute pubescentia, marginibus integris ("rarius serrata" ex Karst. et Schz. Bip.) et in specimine sicco subrevolutis; foliolo terminale lanceolato, 1–1.8 cm. longo; foliolis lateralibus oblanceolatis aut obovatis, 0.6–1.3 cm. longis; petiolo ciliato, 1–1.8 cm. long. Capitula pedunculata, ligulata, circiter 1 cm. lata. Involucrum basi hispidum; squamis duplici serie dispositis et subaequalibus; exterioribus circiter 5, linearibus, plus minusve pubescentibus, nigro-striatis, 3–4 mm. longis; interioribus

lanceolatis, subglabris. Ligulae circiter 6 aut 7, flavae, nigrostriatae nonnullis lineis, 5–8 mm. longae. Paleae lineares, striatae, marginibus diaphanis. Achaenia linearia, biaristata aristis retrorsum hamosis, circiter 1 cm. longa.

Karsten, Bogota, Columbia (type in Herb. Mus. Hist. Nat. Paris).

This species appears very unique, being distinct in general aspect from any other species known to me. The type sheet, originally in SCHULTZ BIPONTINUS' own herbarium, bears several small specimens. These are remarkably uniform and are allied most nearly with *Bidens rubifolia* H. B. K., the leaves of which are much less diminutive. The label bearing the specific name here set forth has also the date Dec. 18, 1856, which, from its position on the label, may be that of the determination rather than the collection. While true *Bidens rubifolia* grows at Bogota (e.g., *I. F. Holton* 365, Oct. 28, 1852), KARSTEN and SCHULTZ BIPONTINUS evidently regarded the Karsten plant specifically distinct. This course I also must adopt, as I am unable to connect the two forms in herbaria by any intermediate forms.

BIDENS ODORATA Cav. Icon. et Descript. Plant **1**:9. *pl. 13*. 1791; *Coreopsis ferulaefolia* Jacq. var. *odoratissima* Pers. Synops. Plant. **2**:477. 1807; *Bidens ferulaefolia* DC. var. *odoratissima* DC. Prodr. **5**:603. 1836. For many years, PERSOON'S variety *odoratissima* of *Coreopsis ferulaefolia* seems to have been unfamiliar to botanists. DECANDOLLE (*l. c.*) merely mentioned it: "quid sit var. *odoratissima* à Persoonio citata ignoro." PERSOON'S variety was founded on CAVANILLES' plant in JUSSIEU'S herbarium and was said to be native to Peru. This cited specimen I have not found, but there exists in the British Museum a good specimen from CAVANILLES, labeled "*Coreop[s]is odoratissima* nobis, Mexico," which matches PERSOON'S description precisely. The leaves, probably because of having grown under cultivation, are especially slender-divided and give a superficial resemblance to those of *Bidens ferulaefolia* (Jacq.) DC. The specimen is identical with two other specimens (in Herb. Brit. Mus.) from the herbarium of CAVANILLES, one labeled "*Coreopsis odorata* olim *Bidens*," and the other "*Bidens odorata* Cav. Ic. V. 1, nunc *Coreopsis*." Both of these last two are further labeled, in pencil, "*Cosmos odoratus*."

A study of other mature specimens of *Bidens odorata* shows that it is a Mexican species primarily (if not indeed exclusively), and is not at all a *Coreopsis*, but rather a true *Bidens*, although having achenes sometimes approaching those of *Cosmos* and with rays varying, in herbarium specimens, from yellow to white, rose, or violet (a single sheet frequently showing all these variations, as, for example, *Palmer* 674, Herb. Kew).

During the past few years, certain writers, notably OLIVER and HIERN, S. L. MOORE, and O. HOFFMANN, have described a considerable number of striking and positively new species of *Coreopsis* and *Bidens* from various parts of Africa. In their generic distinctions, however, they have unfortunately been guided mainly by the direction of the barbs on the awns of the achenes, or, in certain cases, they have had access at the time to immature achenes only. Regarding the latter point, the disadvantage and source of error are at once evident on examining the real distinctions between *Coreopsis* and *Bidens*. As will be seen below, the presence of two more or less conspicuous lateral wings upon the achenes seems by far the most nearly constant character of *Coreopsis* as distinguished from *Bidens*. But in several species of *Coreopsis* these wings are completely lacking on the immature achenes. Thus, species described from immature specimens as *Bidens* may later prove to be *Coreopsis*. Again, a species erroneously described from immature specimens as *Coreopsis*, because of a recognized resemblance to some particular species of *Coreopsis* and disregarding the absence of wings, must thereafter be treated by botanists as *Coreopsis* until mature material can be obtained to prove its status as true *Bidens*.

Concerning the direction of the barbs on the awns of the achenes, however, a more detailed statement is needed. Until comparatively recent times, botanists referred to *Bidens* those species with retrorsely barbed awns, and to *Coreopsis* those species with antrorsely barbed awns or with awns inconspicuous. But, from time to time in North America, new forms have been discovered, identical in each case with a certain species of *Coreopsis* (as then delimited) or *Bidens* except in the direction of the barbs on the awns. ASA GRAY (cf. FERNALD, Rhodora **15**:77. 1913), when confronted with such a form of "*Coreopsis aristosa* Michx." (the then accepted name), designated it "*C. aristosa* in Bidentem transformata." Later (Synop. Fl. N. Amer. 1$^{\text{II}}$:294–296. 1884) he treated this and similar forms as hybrids between *Coreopsis* and *Bidens*. Still later, BRITTON (Bull. Torr. Bot. Club **20:** 280 281. 1893), emphasizing the instability of the barb-direction character for *Bidens frondosa* L., and also separating the two genera on general grounds rather than by one particular character, transferred six species from *Coreopsis* to *Bidens*. The validity of these transfers has since been accepted unhesitatingly by all prominent American botanists who have critically studied the Eastern United States species of *Bidens*, among them WIEGAND (Bull. Torr. Bot. Club **26:** 401. 1899), GREENE (e.g., Leafl. Bot. Crit. **1:** 200.[2] 1906), and ROBINSON and FERNALD (GRAY'S Man. ed. 7. p. 839. 1908). It is also implied by many other American botanists working upon the species of other regions but following the same distinctions, a singular case being that of BRANDEGEE'S description (Zoe **5:** 239. 1908) of *Bidens alpina* and GREENMAN'S description (Proc. Amer. Acad. **41:** 264. 1905) of *Bidens sarmentosa*. The achenes are described for *B. alpina* as "nearly smooth; awns none or two varying from 1 mm. long to rudiments, corneous and not barbellate"; for *B. sarmentosa*, as "glabrous or sparingly hispidulous, awnless or with reduced awns." But, on investigation, these species are found to be identical; in turn, *B. sarmentosa*, which from priority of publication would otherwise stand as the accepted species, is found to match[3] in every character *Coreopsis anthemoides* DC., having achenes described (DC. Prodr. **5:** 573. 1836) as "(immaturis) linearibus glabris brevissime bidentatis." Thus, a species placed by DECANDOLLE in *Coreopsis*, evidently because of its very short awns, was independently referred by BRANDEGEE and by GREENMAN, about 70 years later, to *Bidens*, evidently because the achenes lack wings and the general characters coincide closely with those of certain unquestioned species of *Bidens* (e.g., *B. humilis* H. B. K., with awns retrorsely barbed).

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[2] Thus, GREENE refers to *Bidens* a plant ("*B. tenuissima*") with "erect, *upwardly barbed awns*."

[3] I have examined several authentic cotypes of *B. alpina*, the type and numerous authentic cotypes of *B. sarmentosa*, also several cotypes of "*Coreopsis anthemoides*" (*Bidens anthemoides* Sherff, BOT. GAZ. **56:** 493. 1913). War conditions in Europe have precluded for the present my examination of DECANDOLLE'S type at Geneva. However, the characters cited by DECANDOLLE and his comparison of this species with *Anthemis arvensis*, which it at times simulates in habit to a remarkable degree, leave no doubt that the cotypes examined (in Herb. Brit. Mus. and elsewhere) truthfully represent the type.

Fernald, in a recent discussion of the awn characters of *Bidens* (Rhodora **15**:74 78. 1913), lists no less than six American species in which occurs a more or less pronounced form having the awns barbed in the reverse from the normal direction. Thus, for example, *Bidens connata* Muhl. has awns retrorsely barbed, while var. *anomala* Farwell has awns antrorsely barbed. Again, *Bidens aristosa* (Michx.) Britton has antrorsely barbed awns, while the probably valid var. *Fritcheyi* Fernald has retrorsely barbed awns. Hence it is obvious that, were the old artificial method of distinguishing between *Coreopsis* and *Bidens* (namely by the direction of the barbs on the awns) to be retained, an anomalous situation would result. We should be compelled either to regard each of these varieties as a hybrid between two species of distinct genera, a course certainly unwarranted in several cases (cf. Fernald, *l. c.*, and Wiegand, Bull. Torr. Bot. Club **26**:401. 1899), or to refer each variety to the other genus, an entirely indefensible alternative. We are compelled, then, to view these varieties, in at least the majority of cases, as merely more or less distinct and pronounced forms of their respective species. This being true, the awn character method of separating *Coreopsis* from *Bidens* proves utterly worthless, and must be permanently abandoned, as it indeed has been by American botanists.

On reference to recent descriptions and types of African species of *Bidens*, we find that in several cases the awns, even on achenes on the same head, are barbed both antrorsely and retrorsely. Thus, for example, Moore (Jour. Linn. Soc. Bot. **37**:322. 1906) created the name *Bidens ambigua* for *Gossweiler* 1189, precisely for the reason that some of the awns are smooth, others antrorsely barbed, and others retrorsely barbed ("achaeniis . . . . aristis 2 quam se ipsa brevioribus dentibus perpaucis nunc erectis nunc recurvis onustis vel etiam omnino calvis . . . . , hence the trivial name"). Yet in the same year (Jour. Bot. **44**:22. 1906) he likewise somewhat arbitrarily created the name *Coreopsis Taylori* for a plant showing the same variation (coll. *W. E. Taylor*, Jan. 5, 1886;

"achaeniis . . . apice setuloso-ciliatis calvis vel aristulis 1 vel 2 brevissimis erecto—vel patenti—vel etiam recurvo—uncinulatis onustis saepe vero nudis . . . ; indeed, the plant might almost as well be considered a *Bidens*, but the habit is that of *Coreopsis*"). In referring the latter species to *Coreopsis*, he relied mainly upon its habital similarity to other (so-called) species of *Coreopsis* from Africa. But, as will be seen presently, some of these species belong in reality to *Bidens*. Therefore, this habital similarity, affording formerly an apparently good reason for the name *Coreopsis Taylori*, can no longer be given much consideration.

The present writer, in bringing together the numerous species of *Bidens* for monographic treatment, has come to adopt fully the generic limits of these two genera as followed by recent American botanists. In brief, the genus *Coreopsis* is maintained primarily because of the peculiar habit and winged achenes of the Linnaean type species (excluding *C. alba*, *C. Bidens*, and *C. alternifolia*, Sp. Plant ed. 1. pp. 907–909. 1753; cf. BRITTON, Bull. Torr. Bot. Club **20**: 280. 1893). Similarly, the genus *Bidens* is maintained primarily because of the peculiar habit, strongly barbed awns, and wingless achenes of several of the Linnaean type species of *Bidens*. Among the species of either small group a fair degree of uniformity in several characters occurs. But on extending our observations to other species of *Coreopsis* and *Bidens*, we find remaining no absolute uniformity in even one distinctive character. However, one such character does persist to a surprising extent. It is the presence (in *Coreopsis*) or absence (in *Bidens*) of two lateral wings upon the mature achene. Among so many species from widely remote regions does this character separate two genera with different aspects that, *in cases where other criteria are absent*, it appears to offer the only logical basis of distinction. Accordingly, and with a view to thus delimiting these two genera more accurately, notably among the African species (where the generic limits tend to overlap) this basis of distinction is here adopted. As a consequence, it is found necessary to make the following transfers for the flora of Africa. This list includes only those species upon the types or other positively authentic material of which I have personally examined the mature achenes.

Bidens arenicola (S. Moore), comb. nov.—*Coreopsis arenicola* S. Moore, Jour. Linn. Soc. **37**:170. 1905.

Bidens Grantii (Oliver), comb. nov.—*Coreopsis Grantii* Oliver, Trans. Linn. Soc. **29**:98. *pl. 65*. 1873.

Bidens grandis, nom. nov.—*Coreopsis speciosa* Hiern, Cat. Welw. Afr. Pl. **3**:585. 1898.

Bidens Kirkii (Oliver and Hiern), comb. nov.—*Coreopsis Kirkii* Oliver and Hiern, Fl. Afr. Trop. **3**:390. 1877.

The type material (in Herb. Kew) lacks mature achenes, but the mature material of *G. F. Scott Elliot* 6909, referable to this species, has the achenes of a true *Bidens*.

Bidens ambacensis (Hiern), comb. nov. -*Coreopsis ambacensis* Hiern, Cat. Welw. Afr. Pl. **3**:586. 1898.

Bidens ugandensis (S. Moore), comb. nov.—*Coreopsis ugandensis* S. Moore, Jour. Linn. Soc. **35**:347. 1902.

Bidens ruwenzoriensis (S. Moore), comb. nov.—*Coreopsis ruwenzoriensis* S. Moore, Jour. Linn. Soc. **35**:345. 1902.

Bidens kilimandscharica (O. Hoffm.), comb. nov.—*Coreopsis kilmandscharica* O. Hoffm., Bot. Jahrb. **20**:234. 1894.

Bidens Schweinfurthii, nom. nov.—*Coreopsis linearifolia* Oliver and Hiern, Fl. Afr. Trop. **3**:390. 1877.

This species is here given a new name to avoid any possible confusion with *Bidens linearifolia* Schz. Bip., which, in fact, is a true *Cosmos* and has been renamed correctly *C. linearifolius* Hemsley (Biol. Centr. Amer. 2:200. 1881).

Bidens Taylori (S. Moore), comb. nov. *Coreopsis Taylori* S. Moore, Jour. Bot. **44**:22. 1906.

Bidens insecta (S. Moore), comb. nov. -*Coreopsis insecta* S. Moore, Jour. Bot. **46**:42. 1908.

Bidens Baumii (O. Hoffm.), comb. nov.—*Coreopsis Baumii* O. Hoffm. in H. Baum Kun.-Sambes. Exped. 419. 1903.

Bidens Elliotii (S. Moore), comb. nov. -*Coreopsis Elliotii* S. Moore, Jour. Linn. Soc. **35**:346. 1902.

BIDENS FLORIBUNDA H. B. K., Nov. Gen. **4**:238. 1820; *Bidens simplicifolia* Wright, Kew Bull. 1906: 5. 1906. —The type of *B. simiplicifolia* Wright (in Herb. Kew) is identical with the type specimen of *B. floribunda* H. B. K. (in Herb. Mus. Hist. Nat. Paris).[4] An important feature of *B. floribunda* is its simple leaves, although KUNTH, in the original description, betrayed doubt as to whether tripartite leaves are completely lacking (foliis . . . simplicibus, nisi folia inferiora, a me haud visa, in hac quoque specie ternata sint"). WRIGHT, in describing *B. simplicifolia* ("a speciebus reliquis Austro-Americanis foliis indivisis ovatis acuminatis differt"), was clearly unaware that this same species had already long before been described from the same country (Ecuador) as *B. floribunda*. An excellent cotype of *B. simplicifolia* in the Herbarium of Field Museum has foliage much superior to that of the type. It possesses four pairs of large simple leaves, the lowermost ones 15.2 cm. long and 5.6 cm. wide. From these it would appear even more plausible that the species is constantly simple leaved.

BIDENS ALAUSENSIS H. B. K., Nov. Gen. 4:184. 1820; *Bidens valparadisiaca* Colla, Mem. Accad. Torin. **38**:12. *pl. 24.* 1835; *Bidens chilensis* DC., Prodr. **5**:603. 1836. A study of several authentic specimens of *B. chilensis* DC. collected around Quillota, Chile, by BERTERO about 1829, shows that these are precisely the same as the type specimen (fig. 1) and BONPLAND's private duplicate specimen of *B. alausensis* H. B. K. (both specimens in Herb. Mus. Hist. Nat. Paris). Other specimens, collected at various dates by GAY, W. H. HARVEY, BRIDGES, etc., and all determined as *B. chilensis*, show that the rays are frequently white, as stated by BERTERO (DC., *l. c.*), instead of yellow, as in DECANDOLLE'S type specimen.

Regarding *B. valparadisiaca* Colla we need only to say that it was founded upon BERTERO's material, as was DECANDOLLES *B.*

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[4] The only difference that I can detect is that, in at least its cotype material examined, *B. simplicifolia* has the exterior involucral bracts mainly subspatulate and only slightly ciliate; in the type of *B. floribunda*, these are more oblong and more ciliate. While such a variation in shape or size of these bracts has already, in some species, been made the basis for a varietal distinction (e.g., *B. rosea aequisquama* Fernald, Proc. Amer. Acad. **43**:68. 1907), such a course would seem undesirable here. Aside from the fact that these bract characters frequently vary, in other better known species of *Bidens*, from oblong to spatulate on the same head, it would mean the unwelcome use, according to rules, of the name *simplicifolia* for a variety of a species that itself is simple leaved (*simplicifolia*).

chilensis,[5] and hence likewise must be synonomized with *B. alausensis*.

It is interesting to note that *Bidens crithmifolia* H. B. K. was suspected by SPRENGEL (Syst. Veg. ed. 16^{III}. p. 454. 1826) of belonging to *B. alausensis*. A study of the types of both species shows, however, that they are entirely distinct.

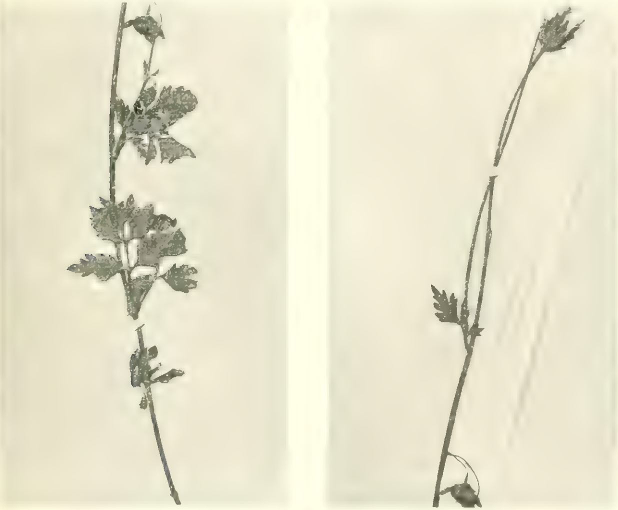

FIG. 1.—Type of *Bidens alausensis* H. B. K. in Herb. Mus. Hist. Nat. Paris; the two photographs slightly overlap.

BIDENS TENELLA L., Amoen. Acad. **6**:96. 1763. This species, was included by DECANDOLLE (Prodr. **5**:605. 1836) among the "species non satis notae." A sheet with two specimens exists however, in the Linnaean Herbarium. These are in good condition and show at once that the species is in no way a *Bidens*, and may even be founded upon two distinct plants from widely separate parts of the world. In fact, the sheet bears a determination by SCHULTZ BIPONTINUS, naming the plant "ad sinistram *Pectis* (*tenella*)" and that "ad dextram Charieis *heterophylla* Cass."

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[5] *B. valparadisiaca* was based (COLLA, *l. c.*) upon a specimen from Valparaiso, while *B. chilensis*, which DECANDOLLE himself equated with it, was based upon a specimen collected in the immediate vicinity, but to the northeast ("circa Quillota," DC., *l. c.*).

BIDENS ANDONGENSIS Hiern, Cat. Welw. Afr. Pl. **1**$^{\text{III}}$:588. 1898. — At the time of describing this species, HIERN confessed himself uncertain as to its generic status, owing to the insufficient material. Since then, however, an admirable, well developed specimen (*John Gossweiler* 3631, Angola, August 3, 1907) has been received at the Herbarium of the British Museum. This matches sufficiently in each detail the plant fragment and drawings on the type sheet, at the same herbarium, and proves conclusively that the species is a true *Bidens*.

Bidens elata, comb. nov.—*Bidens cernua* L. var. *elata* Torr. and Gr., Fl. N. Amer. **2**:352. 1842; *Bidens dentata* Wieg., Bull. Torr. Bot. Club **26**:412. 1899, non *Bidens quadriaristata* DC. var. *dentata* Nutt.; *Bidens amplissima* E. L. Greene, Pittonia **4**:268. 1901. —An excellent specimen of this species, collected by SCOULER at the Straits of De Fuca, is in the Torrey Herbarium, now included in the Herbarium of the N.Y. Bot. Garden. It is identical with the Scouler specimen of HOOKER's herbarium (now in Kew Herb.), a specimen referred by HOOKER (Hook. Fl. Bor. Amer. **1**:314. 1833) to *B. chrysanthemoides* Michx. (but entirely distinct from Michaux's two type specimens in Herb. Mus. Hist. Nat. Paris). It is identical also with the type and cotype specimens of *B. amplissima* Greene. However, it is very different from the type (fig. 2) of *B. quadriaristata* DC. var. *dentata* Nutt. (in Herb. Brit. Mus.), a plant cited synonomously by TORREY and GRAY, but probably never seen by them, as indeed their failure to use their customary exclamation marks would partly imply.

WIEGAND, following TORREY and GRAY'S treatment, likewise equated these two plants, but GREENE (*l. c.*), who, however, had not seen NUTTALL'S type, justly denied their identity. Still GREENE'S name *B. amplissima* is superfluous according to rule of nomenclature, and the name *elata*, supported by a description ("leaves . . . . unequally and incisely serrate," etc., Torr. and

Gr., *l. c.*) that very definitely characterizes the cited specimens,[6] must be used for this species.

Bidens aurea, comb. nov. *Coreopsis aurea* Ait., Hort. Kew. **3**: 252. 1789; *Bidens coronata* auct., non L. In a former article (Bot. Gaz. **56**:495. 1913), it was stated that Britton and not Fischer was the one to transfer *Coreopsis coronata* L. to *Bidens*. An inspection, furthermore, of Britton's article cited (Bull. Torr.

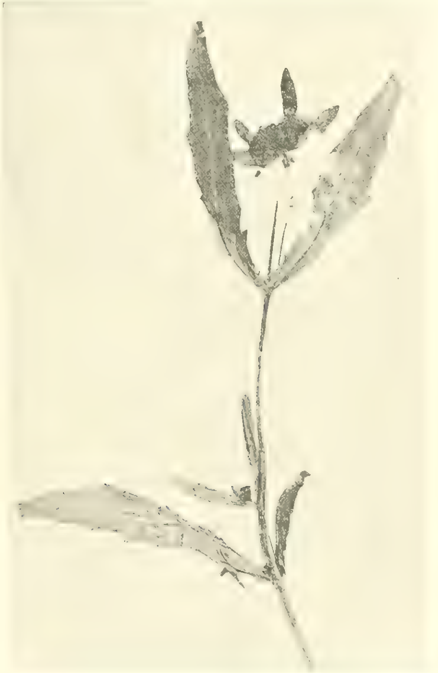

Fig. 2.—Type of *Bidens quadriaristata* DC. var. *dentata* Nutt., in Herb. Brit. Mus.

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[6] At least the Scouler specimens. I have not seen the Douglas specimens.

Bot. Club **20:**280. 1893) shows that he was merely treating a group of *Coreopsis* species of GRAY'S *Synoptical flora* en masse, and was concerned with their generic rather than specific status. And study of GRAY'S descriptions and notes (GRAY, Synop. Fl. N. Amer. **I**$^{\text{II}}$: 294. 1884) shows that GRAY equated, though somewhat provisionally, *Coreopsis aurea* Ait. with *C. coronata* of the Linnean Herbarium, a view retained as well by BRITTON (BRITTON and BROWN, Illustr. Fl. **3:**498. 1913).

My search through the material of *Bidens* and nearly allied genera at Kew Herbarium failed to reveal an original specimen of *Coreopsis aurea* Ait., but a good and authentic sheet from Kew Gardens, in 1785, occurs in the Herbarium of the British Museum. It is the form recently treated by authors as *Bidens coronata* (e.g., BRITTON and BROWN, *l. c.*). In the Linnaean Herbarium, moreover, there still exists the original superb specimen of *Coreopsis coronata* L. It lacks mature achenes, but its several beautiful 8-rayed heads, with the rays strikingly well arranged on the paper (fig. 3), leave no doubt that LINNAEUS had this specimen at hand when describing *C. coronata* (Sp. Plant. ed. 2. **2:**1281. 1763; "radio magno, octopetalo," etc.). While, indeed, LINNAEUS cited in his synonomy plants of VAILLANT and of PLUMMIER, these have been justly excluded by subsequent authors. Thus, for forming a true conception of *C. coronata* L., there are left for us the Linnaean specimen and description. The latter, by itself, is inadequate. The former, in GRAY'S time, seemed likewise disappointing, as being too nearly intermediate between *C. aurea* Ait. and *C. trichosperma* Michx. of his *Synoptical flora*. But, in later years, numerous specimens of these last two species have been added to our American herbaria and show very clearly differences in leaf outlines that GRAY, with his scantier material, could not properly define. A comparison with these specimens shows at once that the Linnaean type is the *Coreopsis trichosperma* Gray (*l. c.*), and hence *Bidens trichosperma* Britton.[7]

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[7] Since the foregoing lines were written, Dr. N. L. BRITTON has kindly written me that many years ago he examined the Linnaean type, but, while entertaining doubts as to its true status, felt constrained, for want of achenes, to follow GRAY'S treatment, except as to generic affiliations. However, Dr. M. L. FERNALD has just informed me that GRAY'S fragment at Gray Herbarium, from the Linnaean Herbarium (where certain heads are missing on the single type specimen), "shows perfectly characteristic fruit of *B. trichosperma*, not of *B. coronata* of recent authors," thus confirming my conclusions in a most emphatic way.

My inability to find MICHAUX's specimen of his *Coreopsis trichosperma* in the Michaux collection at Paris (in Herb. Mus. Nat. Hist. Paris) makes it impossible to state definitely whether MICHAUX's specimen was *Bidens aurea* (Ait.), as here introduced, or

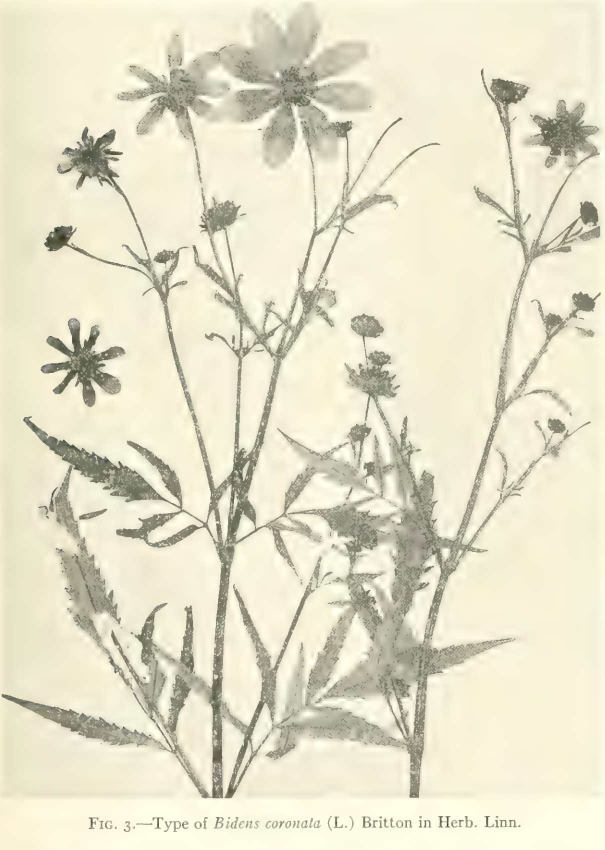

FIG. 3.—Type of *Bidens coronata* (L.) Britton in Herb. Linn.

B. coronata (L.) Britton. Fortunately, lack of priority in either case precludes the use of MICHAUX's name *trichosperma*, and we thus are able to use names —*B. aurea* (Ait.) and *B. coronata* (L.) Britton —that are supported with authentic type or cotype specimens still extant and in a good state of preservation.

The plant with leaves divided into linear segments (*Diodonta leptophylla* Nutt., Trans. Amer. Phil. Soc. II. **7**:360. 1841; *Bidens coronata* var. *leptophylla* Mohr, Contrib. U.S. Nat. Herb. **6**:808. 1901) must properly be designated *Bidens aurea* (Ait.), var. **leptophylla** (Nutt.), comb. nov. NUTTALL's original specimen is still preserved in excellent condition (in Herb. Brit. Mus.).

UNIVERSITY OF CHICAGO

BRANCHING IN THE OPHIOGLOSSACEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 202

(WITH PLATES XX AND XXI AND SIX FIGURES)

LOREN C. PETRY

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LOREN C. PETRY

(WITH PLATES XX AND XXI AND SIX FIGURES)

The occurrence of branching of the rhizome in this family was mentioned by ROEPER (**13**) in 1859, when he described and figured specimens of *Botrychium Lunaria* in which the rhizome bore lateral branches. The origin of such branches was investigated by BRUCHMANN (**2**), who concluded that they were from adventitious buds of superficial origin. FARMER and FREEMAN (**4**) had already ascribed the occasional monopodial branching of *Helminthostachys* to the occurrence of adventitious buds. In 1902, GWYNNE-VAUGHAN (**5**) pointed out that small conical masses of parenchyma occur regularly in the axils of the leaves in this genus and suggested that these are vestigial buds. This view has been confirmed by LANG (**8**), who found that the branches are always axillary in position. The same writer (**9**) has also shown the regular occurrence of similar vestigial buds in the axils of the leaves in *B. Lunaria*, and has demonstrated that the branches of the rhizome arise from these, and not from buds of adventitious origin, as stated by BRUCHMANN.

A branching rhizome of *Ophioglossum vulgatum* was figured by STENZEL (**14**) in 1858; although no statement was made, the figure clearly indicates that the branching is dichotomous. VAN TIEGHEM (**15**) reported similar specimens and, disregarding ROEPER'S figures, stated that all branching of the rhizome in this family is dichotomous. This view of the character of the branching was confirmed for *O. vulgatum* by POIRAULT (11).

The occurrence of the two methods of branching within this family has suggested the desirability of further examination of this feature. This investigation has accordingly been undertaken with a view to securing further data bearing upon the relation of the three genera to each other and of the family to other Pteridophytes.

Ophioglossum

POIRAULT (11) made the first critical examination of the nature of the branching of *O. vulgatum*. He concluded that the branching is usually only apparent, and that it is due to the development of a stem bud upon a young root before the root has broken through the cortex of the parent rhizome. In a single instance true branching was found, and anatomical examination showed this to be dichotomy. So far as the writer is aware, there is no record of branched rhizomes in other species of this genus.

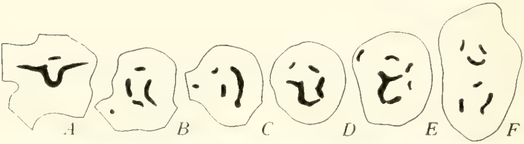

FIG. 1.—Transverse sections of a branching rhizome of *Ophioglossum vulgatum;* only the xylem is shown; ×7.

Ophioglossum vulgatum. In the examination of some 300 specimens of this species, five branched rhizomes were found. These were examined in serial sections, and in all cases the original stele has divided into two equal and similar steles (fig. 1). There can be no doubt that this is dichotomy, as contrasted with monopodial branching. The term "dichotomy" is here used to denote that branching in which a stem divides into two equal stems, as in *Lycopodium*, and is not meant to imply an exactly equal division of the apical cell of the rhizome.

Ophioglossum pendulum. In the examination of some 100 rhizomes of this species, two branched specimens were found. As in *O. vulgatum*, the branching is dichotomous, and the two branches in each case showed an almost exact equality.

In the examination of the material of these two species of *Ophioglossum*, careful search was made for evidence of axillary buds, such as have been described for *Helminthostachys* by GWYNNE-VAUGHAN (**5**), and for *Botrychium Lunaria* by LANG (**9**). No such structures have been found in any case; neither is there any evidence of adventitious budding except upon roots. From this it may be concluded that the true branching of these species is always dichotomous.

Helminthostachys

LANG (**8**) has briefly described the anatomy of two branching rhizomes of this genus. In both, the branches are definitely axillary in position, and it is certain that they have developed from the vestigial buds described by GYWNNE-VAUGHAN (**5**). In each case, the vascular supply of the branch comes from a mass of accessory xylem which develops outside the usual xylem of the stele, either locally at the base of the branch, or surrounding the stele. There is no connection between the branch stele and the subtending leaf trace.[1]

Material of *Helminthostachys* has not been available during the progress of this investigation.

Botrychium

THE OCCURRENCE OF AXILLARY BUDS

All the investigation of branching and of the occurrence of buds has been confined to examination of *B. Lunaria*. On this account, it has seemed advisable to examine other species of this genus with regard to these points. Rhizomes of five species have been secured with branching specimens representing four of these species.

The genus *Botrychium*, as organized by PRANTL (**12**), consists of two sections: EUBOTRYCHIUM, with five species; and PHYLLOTRICHIUM, with eleven species. The latter section is divided into

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[1] The full description of this material has just been published (LANG, WM. H., Studies in the morphology and anatomy of the Ophioglossaceae. III. On the anatomy and branching of the rhizome of *Helminthostachys zeylanica*. Ann. Botany **29**:1–54. *pls. 1–3. figs. 1–8.* 1915). In this paper LANG concludes that although no regular cambium is present, "the development of this accessory xylem should rightly come under the conception of secondary thickening."

two subsections: TERNATA, of eight species; and CICUTARIA, of three species. The material investigated consists of rhizomes of *B. ramosum* and *B. lanceolatum* var. *angustisegmentum*, of the section EUBOTRYCHIUM, to which *B. Lunaria* also belongs; of *B. obliquum* and *B. ternatum* var. *intermedium*, of the subsection TERNATA; and of *B. virginianum*, of the subsection CICUTARIA.

Rhizomes of these five species were examined in serial sections for the presence of such axillary buds as have been described by LANG. In all these species, such buds are uniformly present in rhizomes of all ages, and it may be assumed that they are of constant occurrence in all species of the genus. Their origin and development will be considered later.

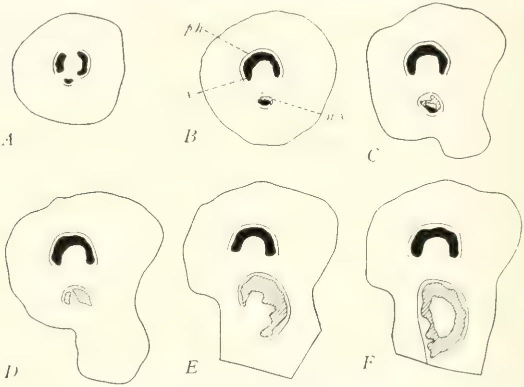

FIG. 2.—Transverse sections of a branching rhizome of *Botrychium lanceolatum* var. *angustisegmentum*: *x*, original xylem of the stem and leaf trace; *nx*, xylem formed after the injury; *ph*, phloem; ×12.

VASCULAR CONNECTIONS OF THE BRANCHES

ROEPER'S (13) figures represent rhizomes of *B. Lunaria* with 2–7 lateral branches. HOLLE (6) figured a single branching specimen of the same species in longitudinal section, and pointed out that in this case the branch stele connects with the trace of the leaf immediately below. LANG (9) has given in detail the vascular connections of the branches in five specimens of this species; he concluded that "the chief vascular supply of the branch is derived from a development of xylem adaxially to the subtending leaf trace." This adaxial xylem is considered to be an extension of the margins of the leaf trace, and therefore centrifugal in character.

Botrychium lanceolatum var. *angustisegmentum*. A single branching specimen of this species was secured. The terminal bud has been destroyed and the branch has arisen at a considerable distance below. As shown by fig. 2, the vascular system of the branch connects with the subtending leaf trace. After the trace has been separated from the stem stele for a considerable distance, tracheids appear on the adaxial side of the protoxylem of the trace; as shown by fig. 3, these are definitely centripetal in origin and occur in contact with the protoxylem. The mass of xylem formed in this way gives rise to the branch stele, which soon assumes the characters of the main stele. This differs from the general condition in *B. Lunaria*, as described by LANG, in that the branch connection arises from centripetal xylem of the leaf trace rather than from adaxial extension of the centrifugal xylem.

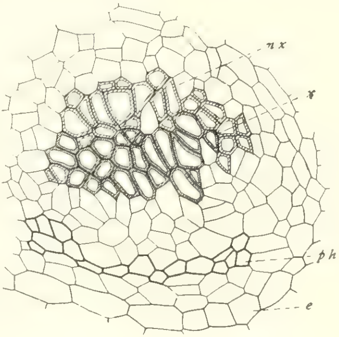

FIG. 3.—Detail of leaf trace of fig. 2, *B*: *e*, endodermis; ×157.

Botrychium ramosum. Two rhizomes of this species, each bearing a single branch, were secured. In each case the terminal bud has been destroyed and the branch has developed at a considerable distance below. The vascular connections of the branches differ greatly from that described above.

Fig. 4 shows the vascular supply of the branch in the first of these specimens. As the leaf trace swings away from the stem stele, wings of primary and secondary xylem develop in the angle between the two (figs. 4, *B*, and 7). At first these extend the entire distance between the two and give the appearance of expansion of the stele in the plane of the leaf trace; but later they separate from the stem stele, as shown by fig. 4, *C*. These wings may be considered to be adaxial extensions of the centrifugal xylem of the leaf trace; there is at no point any evidence of centripetal xylem. Soon after the separation from the stem stele, the leaf trace disappears, having been cut off by the absciss layer and carried out by periderm formation. This leaves two distinct wings of xylem and *each* of these by extension of its margins rounds up into a definite branch stele, as shown in figs. 4, *D*, *E*, *F*, and 8. One of these branch steles soon disappears, having apparently been unable to meet the competition; its apical region has been entirely obliterated by periderm formation. There can be little doubt that both these branch steles developed from a single axillary bud.

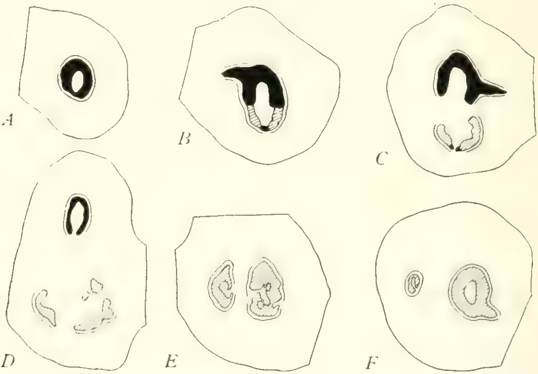

FIG. 4.—Transverse sections of a branching rhizome of *Botrychium ramosum;* ×12.

In the other branching specimen of this species, a wing of xylem extends between the leaf trace and the stem stele on one side only. It separates from the stem stele and at a slightly higher point the leaf trace disappears. By extension of its margins, the wing of xylem rounds up to form a single branch stele. This is exactly the behavior of each of the two wings in the first specimen.

The vascular supply of the branch is here derived in part, at least, from adaxial extension of the centrifugal xylem of the leaf trace, as in *B. Lunaria;* but the formation of either one or two wings of xylem and the consequent formation of one or two branch steles is unique. The significance of this will be discussed later.

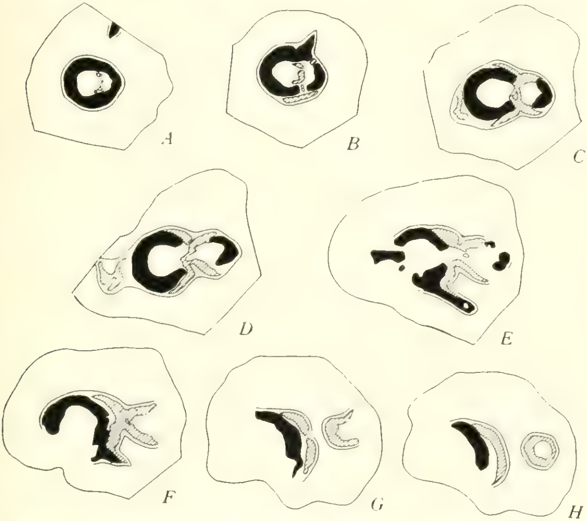

FIG. 5.—Transverse sections of a branching rhizome of *Botrychium virginianum;* ×7.

Botrychium virginianum.—Two branching rhizomes of this species were examined; one of these bore two branches, the other a single one. Fig. 5 shows the vascular connections of the branch in one of these; the injury occurred at the side of the stem and destroyed almost the entire stele (fig. 5, *G, H*). As indicated by the figure, there is a development of xylem in the space between the outgoing leaf trace and the stem stele, as in *B. ramosum;* but here both wings contribute to the formation of a single branch stele. Also procambium strands arise in the pith of the stem opposite the leaf trace (fig. 5, *A*, *B*); these develop a cambium directed toward the center of the stem, which produces a small amount of centripetal secondary wood which also contributes to the vascular supply of the branch. But the greater part of the vascular supply is furnished by the extra or accessory secondary xylem produced by the renewed activity of the cambium (fig. 5, *C*). In this case, therefore, the branch connection consists of (1) accessory secondary xylem, (2) adaxial extensions of the centrifugal xylem of the leaf trace, and (3) a small mass of centripetal secondary xylem originating within the pith of the leaf gap.

One of the other branches shows almost exactly the same connection, but the third branch shows a somewhat different situation. In it the vascular supply is composed principally of accessory secondary xylem; but a small mass of xylem appears in the pericycle of the stem and contributes to the supply of the branch. This pericyclic xylem is added to by a cambium directed toward the phloem; hence we may speak of primary and secondary pericyclic xylem, using the terms "primary" and "secondary" only to indicate the presence or absence of a definite cambium. The vascular supply of this branch, therefore, consists of (1) accessory secondary xylem and (2) primary and secondary pericyclic xylem.

Botrychium obliquum. Four branching rhizomes of this species were secured; three of these bore two branches each, and the other had three branches. In all but one of these, the apical region had been destroyed; but in this one case the rhizome had been injured at the side below the apical region, but without destroying the entire stele. In all the other cases, the branches developed near the apical region. The vascular connection of the lowest of the three branches mentioned above is shown by fig. 6.

As shown by fig. 6, *A*, a very large development of accessory secondary xylem occurs entirely around the stele before the leaf trace separates; at the same level, a cambium has formed within the pith opposite the leaf trace and has developed a considerable mass of centripetal secondary xylem. The injury which occurred at the side of the stele at a slightly higher level has resulted in the destruction of the greater portion of the original xylem of the stem; the attendant periderm formation has produced a considerable distortion of the stele. As the leaf trace separates from the stele (fig. 6, *C*) it is surrounded by a ring of xylem composed on the outer side of accessory secondary wood and on the inner side of centripetal secondary wood. This ring splits into two masses (fig. 6, *D*), and the leaf trace is cut off and carried out by periderm formation. At a somewhat higher level, the accessory secondary xylem closes across the gap (fig. 6, *F*).

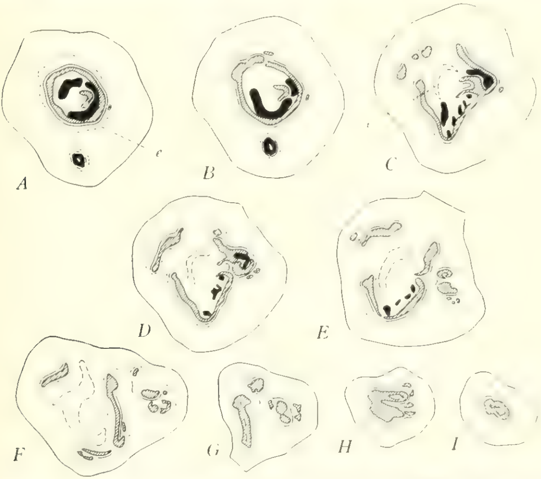

FIG. 6.—Transverse sections of a branching rhizome of *Botrychium obliquum*: *i*, injured region; ×3.5.

At a lower level (fig. 6, *E*), masses of xylem arise in the pericycle of one of the strands which formed part of the ring surrounding the leaf trace. Similar masses of pericyclic xylem occur outside the accessory secondary wood which closed the leaf gap (fig. 6, *F*). The development at this point results in the formation of a closed ring of xylem with cambium and phloem on the inside (fig. 6, *G*); at a slightly higher level, this becomes a tangled mass of tracheids with occasional scattered sieve tubes and parenchyma cells. All these strands—the two produced by the splitting of the ring of xylem about the leaf trace, the strands which arise in the pericycle of one of those, and the tangled mass which is both accessory and pericyclic in origin—fuse to form the branch stele (fig. 6, *H*). Hence the vascular supply of the branch consists of (1) centripetal secondary xylem, (2) accessory secondary xylem, and (3) primary and secondary pericyclic xylem.

In other cases, numerous scattered tracheids appear in the pith below the point of separation of the leaf trace (fig. 9). As the trace leaves the stele, some of these swing out with it and contribute to the vascular supply of the branch. In such cases, there is not the slightest evidence of an internal cambium. Primary centripetal xylem of this kind occurs in the vascular supply of five of the nine branches, while abundant centripetal secondary wood is present in the other four.

It is not necessary to describe in detail the vascular connections of the other branches. Accessory secondary wood usually forms a considerable part of the supply and is present in every case. In six of the nine branches, wings of xylem form between the leaf and the trace and the stele, as in *B. ramosum;* in the other three such formations are entirely lacking. Xylem of pericyclic origin makes up a part of the connection in five cases; in three of these a definite cambium is present. The accessory secondary wood is the only formation which occurs in all the nine cases of this species.

The significance of the wide variation in the vascular supply of the branches will be discussed later.

WOUND REACTIONS

JEFFREY (**7**) has put forward the view that traumatic reactions are apt to be reversionary in character. BOWER (**1**) and LANG (**9**) have used this view in their contention that the pith of the Ophioglossaceae is stelar in character. In the examination of branching specimens of *Botrychium*, especially of *B. obliquum*, some further data upon the nature of the wound reactions of this genus have been secured.

Periderm formation. The formation of cork at the point of injury has commonly been accepted as a direct response to conditions. In several of the rhizomes of *B. obliquum*, injuries affected all the tissues of the stem. In these cases, periderm is formed by every tissue that is still capable of growth; that is, by cortex, endodermis, pericycle, cambium, and pith. Periderm formation by the pith is shown in fig. 10.

The formation of vascular tissue by the pith. In three specimens of *B. obliquum*, tracheids scattered through the pith were found in considerable numbers; fig. 9 shows a section through such a stem. In these cases the injury is at some distance above the point of appearance of the pith tracheids.

In both branching specimens of *B. virginianum* and in three of the four of *B. obliquum*, a considerable development of secondary xylem occurs within the pith; figs. 10 and 11 show examples of this. In all cases this development of centripetal secondary xylem occurs below and opposite the point of departure of a leaf trace. The cambium is always directed toward the center of the stele; well developed sieve tubes are present in most cases.

The formation of vascular tissues by the cambium.—In every injured rhizome of *B. virginianum* and *B. obliquum* a renewed activity of the cambium has occurred. Lignification does not always take place in the first elements produced by this renewed activity; this produces a narrow strip of cells of rectangular cross-section just outside the original xylem of the stem. Usually, however, tracheids resembling those of the original growth are produced by this renewed cambial activity (fig. 10); these make up the accessory secondary xylem mentioned above. It is to be noted that no such accessory xylem was found in either of the two species of the section EUBOTRYCHIUM that were examined, but that such formation occurs in *B. Lunaria* as reported by LANG.

The formation of vascular tissues by the pericycle. In every injured rhizome of *B. virginianum* and *B. obliquum* the pericycle has produced xylem in greater or less amount. As already stated, these masses of pericyclic xylem sometimes contribute to the vascular supply of the branches. When the amount of xylem that occurs is relatively small, only a tangled mass of tracheids is formed; but frequently a cambium develops and produces tracheids in definite radial rows (fig. 12). The cambium usually occurs on the inner side of the xylem, but in a few cases it is located on the outer side, and sieve tubes occur between it and the endodermis.

The endodermis.—A well marked external endodermis is constantly present in all species of *Botrychium* that were examined. The suberized band on the radial walls is particularly heavy in *B. virginianum* and *B. obliquum*. In uninjured rhizomes of these two species, the external endodermis is continuous except at the points of departure of leaf traces. As a trace swings out from the stele, the endodermis breaks at the side of the trace and closes again rather higher up across the leaf gap; the endodermis on the abaxial side of the trace persists for a short distance only. In injured rhizomes of these two species, the irregular growth of the various stelar tissues has produced distortions and breaks of the endodermal layer (fig. 6). In these specimens, the cells of the endodermis frequently divide by periclinal walls; but there is no evidence that vascular elements are ever formed as a result of this growth. In no case were vascular elements found definitely outside the external endodermis. The only internal endodermis found was that occasionally formed by the folding in of the margins of the external endodermis in cases of greatly disturbed steles (fig. 6, C).

Discussion. The occurrence of scattered tracheids in the pith has been observed in *B. ternatum* by BOWER (**1**) and in *B. Lunaria* by LANG (**9**). As stated above, both scattered tracheids and distinct secondary xylem occur frequently in the pith of injured rhizomes of *B. virginianum* and *B. obliquum*. The occurrence of well organized strands of xylem in the pith of a rhizome of *Ophioglossum pendulum* has been reported by the writer (**10**). In view of these facts, it may be concluded that the pith is definitely stelar in character in *Botrychium* and probably in all three genera of the family.

A slight intrusion of cortical tissue might occur at the point of the break of the endodermis without affecting the pith; such an intrusion would affect only the tissues between the leaf trace and the leaf gap. It may be pointed out that in branching specimens of *B. ramosum*, *B. virginianum*, and *B. obliquum*, xylem is produced in quantity at this location; hence this tissue is not an intrusion of the cortex, but is stelar in character. It is to be noted that this tissue is opposite a break in the endodermis, but not outside it.

The view is held that there is a morphological distinction between stelar and cortical tissues; that the suberization of the radial walls of a layer of cells is a physiological phenomenon which under the usual conditions of development occurs in the layer of cells next outside the stele; and that this suberization, under the usual conditions, may be considered an indication of the morphological boundary of the stele, but that it is subject to variation with physiological conditions. LANG has concluded that the internal endodermis of *B. Lunaria* is of physiological significance only, and has suggested that its development is associated with the long leaf gaps of the intermediate region of the rhizome. It is to be noted that the apparent internal endodermis in injured specimens of *B. obliquum* (fig. 6, *E*, *F*) occurs between masses of vascular elements and points of injury.

Of the stelar tissues, the tracheids and sieve tubes are incapable of further growth. The remaining tissues are separated by these into three groups: (1) pith, (2) cambium and adjacent parenchyma, and (3) pericycle. The data given above show that all these three tissues may produce vascular elements, either tracheids or sieve tubes. Hence we may conclude that the production of vascular elements by any stelar tissue is limited only by the capacity of the tissue for further growth.

The manner of that further growth of a stelar tissue varies with the species. In *Ophioglossum pendulum*, which has no secondary thickening, the strands of xylem in the pith of an aberrant specimen were primary in origin. In *Helminthostachys*, no secondary xylem is formed under the usual conditions of development; and renewed growth of the stelar tissues, as in the case of branching, produces an irregular secondary thickening of the stele without a definite meristematic layer. On the other hand, *B. virginianum* and *B. obliquum* show very great development of secondary wood under usual conditions. In both these species, renewed growth of the pith, cambium, and pericycle manifested itself in part by the formation of secondary xylem. Hence we may conclude that the manner of formation of vascular elements by stelar tissues varies with the species, but is relatively constant in any one species under various conditions.

Examination of the various structures shows that they differ mainly in the tissue producing them and in amount of development. That is, these individual variations are differences of position and quantity of vascular elements, and as such their explanation is to be looked for among the physiological factors operating at the time of their development. From this point of view, vascular structures produced as a result of injury may readily show ancestral characters; but such characters are to be considered, not as the repetition of a definite stage of the phylogenetic development of the form, but rather as an indication of the recurrence of certain conditions of development.

It seems well to insist at this point that vascular strands are secondary structures as compared with the tissues which they traverse. The formation of an organ creates a physiological demand to which the vascular strand is a response; and uniformity of the structure which results is only an indication of uniformity of demand and of uniform conditions of development. In this view, the vascular connections of the branches are determined by factors of the same character as those controlling wound reactions.

The vascular supply must be contributed by stelar tissues capable of growth; these tissues are the ones already enumerated, pith, cambium and adjacent parenchyma, and pericycle, together with the parenchyma between the leaf trace and the leaf gap. The manner of formation of the vascular elements of the branch supply is more or less restricted in any species to the particular method of that species. Thus in *B. ramosum*, in which secondary wood formation is relatively slight, no renewed cambial activity occurred in connection with the formation of a branch; while in *B. obliquum*, in which secondary wood formation is very marked under usual conditions, not only does the cambium begin active growth in every case of branching, but similar cambial activity is sometimes set up in both pith and pericycle. The physiological demand likewise varies; in the branch of *B. ramosum* represented in figs. 4 and 8, two growing points were doubtless established. This produced a physiological demand which, operating under the conditioning factors just described, produced two branch steles.

The conclusion that is reached, therefore, is that the vascular connections of the branches are determined in general by three factors. These factors are (1) the presence of stelar tissues capable of growth within the range of the influence of the developing branch; (2) the nature of the growth which can be induced in those tissues, in particular, whether such growth is cambial or not; (3) the physiological demand produced by the growing branch. The first of these factors will vary with the distance of the bud from the leaf trace as compared with its distance from the stem stele; and, more particularly, with the age of those structures when the branch begins to develop. The second factor may be considered to be relatively constant for any one species but to vary widely with different species. The vascular connection produced is the direct result of the third factor acting upon and limited by the other two; the individual conditions give to this third factor a special value for each particular case; the resultant structures are therefore direct responses to unknown and varying conditions and of physiological interest only. It is therefore concluded that the vascular connections of the branches of *Botrychium* have little or no phylogenetic significance.

THE ORIGIN AND DEVELOPMENT OF THE AXILLARY BUDS

In view of the foregoing conclusion, attention has been turned from the anatomy of the branch connections to the examination of the origin of the axillary buds. Since LANG's description of these buds in *B. Lunaria* deals only with the mature structures, the further investigation has been directed toward the examination of the origin and development of these buds in *B. obliquum*.

The apical region. The apical region of *Botrychium* has been described in detail by HOLLE (**6**), CAMPBELL (**3**), and BRUCHMANN (**2**). All agree that growth takes place by means of an apical cell of the form of a triangular pyramid, and that each segment of the apical cell probably gives rise to a leaf. BRUCHMANN states that in *B. Lunaria* leaf formation begins by the appearance of an apical cell within a segment; and that the entire segment rises abruptly above the plane of the apex of the stem.

In connection with the investigation of the origin of the axillary buds, the apical region of *B. obliquum* has been examined, and the results will be given briefly. As shown by figs. 13 and 14, the apical cell is a triangular pyramid, with three cutting faces. The first division of a segment is by a periclinal wall, as stated for *B. virginianum* by CAMPBELL; the further divisions are irregular.

While the limits of the various segments cannot always be exactly defined, it seems certain that each segment gives rise to a leaf; hence one segment is cut off each year. Fig. 13 represents a transverse section through the apical region of a plant collected early in April; the first segment was cut off during the preceding year and has divided transversely. In the second year, irregular divisions, both longitudinal and transverse, take place with the segment; at the end of the year, the segment consists of 6–15 cells. During the third year, this irregular division continues; but there is little or no extension above the plane of the apex. At the beginning of the fourth year, the segment begins a much more rapid growth and rises abruptly above the plane of the apex, as described by BRUCHMANN; at about the same time, an apical cell is recognizable within the segment, and the further growth is definitely apical. It is evident that while the entire segment takes part in the initial growth, the leaf is formed from only a part of the segment; the remainder of the segment builds up the stem tissues. By the end of the fourth year, the leaf has become a hemispherical mass which has grown forward and upward, and its forward margin extends considerably beyond the apical cell of the stem (fig. 14). Early in the fifth year, the fertile spike makes its appearance. It is first recognized as an apical cell on the forward side of the apical cell of the leaf; by the end of the year, it has produced a well defined knoblike structure. During the sixth year, both the fertile and sterile portions of the leaf develop rapidly. In the summer of the seventh year, this leaf breaks through the base of the enveloping older leaf, and the spores are shed in September. The fertile spike then withers, but the sterile portion of the leaf persists through the winter, and only dies in the early summer of the eighth year, after the emergence of the next younger leaf.

The bud.—As stated above, the first evidence of the formation of a new leaf is the abrupt rise of the entire segment above the plane of the apex. This growth is relatively slight in amount, and the further development of the young leaf is apical. The cells produced by the apical growth are arranged in such definite rows (fig. 15) that it is easy to distinguish the tissue produced by the apical cell.

The axillary bud can first be distinguished on the adaxial face of the base of a young leaf, after the apical growth of the leaf has proceeded to a considerable extent (fig. 15). At this time, it consists of a plate of meristematic cells, 6–8 in number; there is no evidence of an apical cell. The arrangement of the surrounding cells shows clearly that the bud has not come from the apical cell of the leaf, but has arisen from cells carried up by the elongation of the segment. By the upgrowth of the apical region of the stem, this plate of meristematic cells is thrown into a crevice, which lies between the stem and the adaxial face of the base of the leaf; fig. 14 shows the appearance of a bud a year older than that shown by fig. 15. It consists of a mass of 30–40 cells, lying as a plate at the base of the leaf; one of the cells shown has divided by a periclinal wall, but such a division occurs rarely.

In older leaves, the location of this crevice or slit is readily pointed out by the margin of the stipular sheath of the next younger leaf; this makes the identification of the buds in longitudinal sections of the rhizome particularly easy. Fig. 16 shows the margin of the stipular sheath of the functioning leaf of a rhizome, and the bud formed in the axil of the leaf of the preceding year; fig. 17 shows a bud a year older. They consist of plates of cells of a meristematic nature; these plates are never more than two cells in thickness and are usually 5–8 cells in longitudinal extent. In tangential sections of a rhizome (figs. 18, 19), they are seen to be 6–10 cells in width; the canal by which they communicate with the exterior is a mere slit. There is not the slightest evidence of any apical cell, or other indication of an apex. They agree in all essentials with the buds of *B. Lunaria* as described by LANG.

Discussion.—As stated above, neither axillary nor adventitious buds occur in the rhizomes of either *Ophioglossum vulgatum* or *O. pendulum;* and where branching occurs in these species, it is dichotomous. On the other hand, the buds and branching of *Helminthostachys* resemble those of *Botrychium* in every respect. These facts may be considered further evidence of the close relationship of these two genera; at the same time, they will serve to emphasize the differences between these two genera on the one hand and *Ophioglossum* on the other.

LANG (9) has mentioned the similarity between the vascular connection of the branches of *Botrychium* and of species of the Hymenophyllaceae. For purposes of comparison, the origin of the axillary buds of a species of *Trichomanes* from Samoa has been examined. The leaf has an apical cell from the beginning and develops to a considerable extent before the branch appears. The formation of the branch is initiated by the appearance of an apical cell. The exact method of formation of this apical cell of the branch was not determined; but it is evident that it arises late and from the growing point of the leaf. The axillary branches of *Trichomanes*, therefore, are foliar in origin. In *Botrychium*, as shown above, the axillary bud is in no way related to the apical cell of the leaf; it arises directly from a portion of a segment of the apical cell of the rhizome; and its position on the base of the leaf is incidental and does not indicate a foliar origin. The similarity between the branching of *Botrychium* and *Trichomanes*, therefore, is not close.

The mature axillary buds of *Botrychium* are of the simplest possible form, an undifferentiated layer of meristematic cells; and it is to be noted that at no time in their development is there any differentiation. This may be accepted as evidence of reduction produced in connection with dormancy; and we may conclude that *Botrychium* and *Helminthostachys* have been derived from a form which branched freely in a monopodial fashion. This is in full agreement with other evidence which points to a relationship of the Ophioglossaceae to the primitive forms of Filicales, especially the Zygopterideae.

Summary

1. Branching of the rhizome of *Ophioglossum vulgatum* and *O. pendulum* is dichotomous; there are no axillary or adventitious buds on the rhizome.

2. Axillary buds are regularly present in five species of *Botrychium.*

3. The vascular connections of the branches in *Botrychium* vary widely with the species and with the individual specimen. It is concluded that the details of the vascular supply of the branch are controlled by the conditions of development and are therefore of little or no phylogenetic importance.

4. In wounded rhizomes of *B. obliquum*, renewed activity of the cambium produces considerable masses of accessory xylem; the pith frequently develops sieve tubes and a cambium which produces secondary xylem in quantity; the pericycle often produces sieve tubes and secondary xylem. It is concluded that in this species any stelar tissues capable of growth may produce vascular elements under the influence of an injury.

5. The axillary bud of *B. obliquum* arises as a plate of meristematic cells on the adaxial face of the base of the very young leaf; it develops without differentiation into a plate of meristematic tissue one or two cells in thickness and 50–60 cells in area, which is buried by overgrowth of surrounding tissue.

6. The data secured is in agreement with the evidence pointing to a relationship of the Ophioglossaceae to the primitive ferns, especially the Zygopterideae.

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The writer wishes to acknowledge the kindness of Professor John M. Coulter in placing the facilities of the Hull Botanical Laboratory at his disposal; and to express his thanks to Dr. W. J.G. Land for material of *Trichomanes*.

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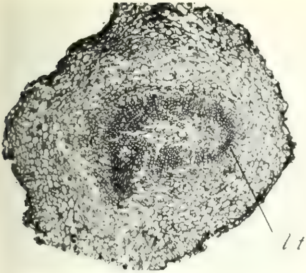
EXPLANATION OF PLATES XX AND XXI

(Figs. 1–6 are in the text)

FIG. 7.—Transverse section of a rhizome of *Botrychium ramosum* at the base of a branch: *lt*, leaf trace; ×20.

FIG. 8.—Transverse section of the branch whose origin is represented by figs. 4 and 7, showing the two branch steles: *a*, larger stele of the branch; *b*, smaller stele of the branch; *r*, trace of root which connects with the smaller branch stele; ×20.



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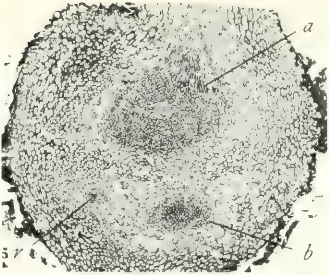

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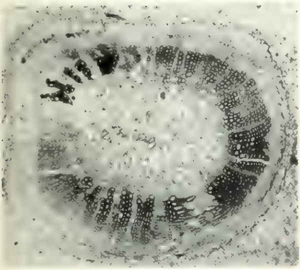

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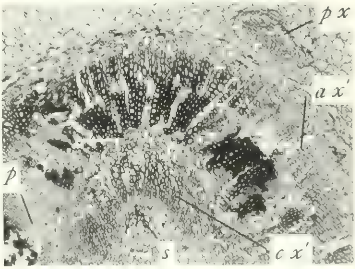

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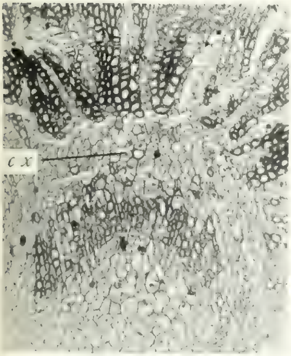

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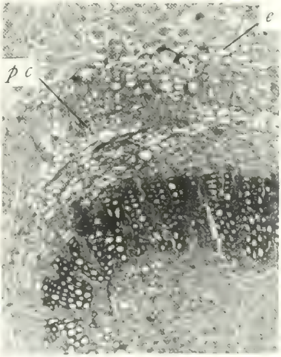

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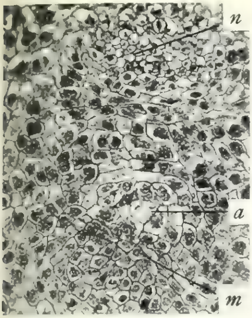
PETRY on OPHIOGLOSSACEAE



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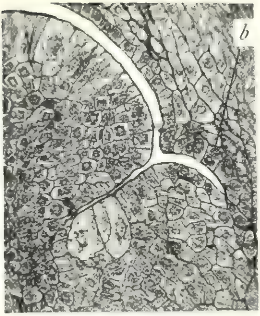

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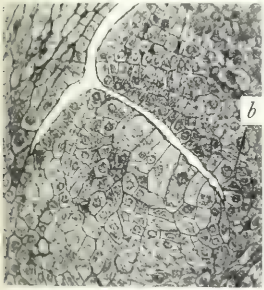

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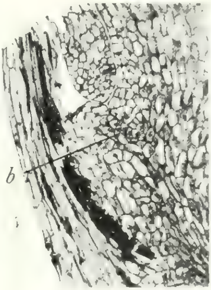

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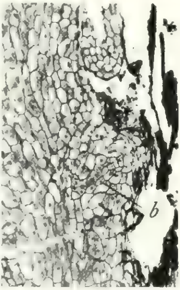

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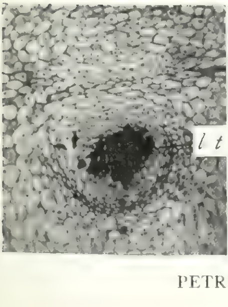

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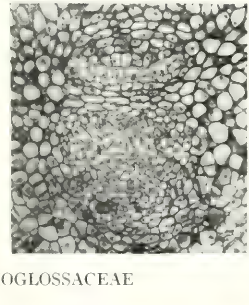

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PETRY on OPHIOGLOSSACEAE

FIG. 9.—Transverse section of a stele of *B. obliquum*, showing tracheids scattered through the pith; ×20.

FIG. 10.—Transverse section of a portion of the stele of an injured rhizome of *B. obliquum*, showing development of new vascular elements in connection with the formation of a branch: *cx′*, centripetal secondary xylem; *ax′*, accessory secondary xylem; *px*, pericyclic xylem; *s*, sieve tubes; *p*, periderm; ×27.

FIG. 11.—Transverse section of a portion of the stele shown in fig. 10, at a lower level, and showing detail of the vascular elements in the pith; *cx*, centripetal primary xylem; ×48.

FIG. 12.—Transverse section of a portion of a stele of *B. virginianum*, showing secondary xylem in the pericycle: *pc*, pericyclic cambium; *e*, endodermis; ×48.

FIG. 13.—Transverse section through the apical region of a rhizome of *B. obliquum: a*, apical cell; *m*, procambium of the youngest leaf; *n*, procambium of the next older leaf; ×122.

FIG. 14.—Longitudinal section through the apical region of a rhizome of *B. obliquum: b*, axillary bud of second leaf; ×122.

FIG. 15.—Longitudinal section through the apical region of the rhizome of *B. obliquum: b*, axillary bud of the youngest leaf; ×122.

FIG. 16.—Longitudinal section through axillary bud of *B. obliquum;* ×48.

FIG. 17.—Longitudinal section of mature bud of *B. obliquum;* ×48.

FIG. 18.—Transverse section of axillary bud near its base, as seen in a tangential section of the rhizome: *lt*, trace of the subtending leaf; ×48.

FIG. 19.—Transverse section of bud near the surface of the rhizome; ×48.

MICROTECHNICAL METHODS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 203

W. J. G. LAND

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MICROTECHNICAL METHODS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 203

W. J. G. LAND

An improved method of replacing the paraffin solvent with paraffin

In paraffin imbedding the almost universal custom when replacing a paraffin solvent with paraffin is to add from time to time small pieces of paraffin to the solvent, until at room temperature no more paraffin is dissolved. Then the container is either placed in a low temperature oven or on an oven of higher temperature and paraffin added until the new saturation point is reached. The container is then placed in an oven having a temperature one or two degrees above the melting point of the paraffin and more paraffin is added. When the last paraffin has melted the mixture is poured off and replaced with pure melted paraffin. The object in repeatedly adding small quantities of paraffin is to prevent a too rapid increase in the density of the xylol-paraffin or whatever solvent is used for paraffin.

Objects to be imbedded, being heavier than xylol, sink to the bottom. Paraffin, which is heavier than xylol, also sinks, with the result that the objects are almost immediately surrounded by a dense layer of dissolved paraffin, thereby defeating the end sought by a gradual addition of paraffin. It has repeatedly come to the notice of the writer that much of the damage to delicate plant tissues takes place during the replacement of the solvent by paraffin. Many otherwise careful workers are particularly careless at this point.

In order to prevent the object from coming into immediate contact with the dense xylol-paraffin solution, a piece of wire gauze is bent in such a manner that it forms a support 2–3 cm. above the object, and xylol sufficient to rise 1–2 mm. above the support is added. The container (a shell or bottle) is then filled with blocks of paraffin, corked, and set aside and not disturbed until the saturation point is reached. The container is next placed on the oven until the new saturation point is reached, next in the oven, and imbedding is proceeded with in the usual manner. Even here, when dealing with delicate plants, the xylol-paraffin is poured off only to the wire gauze and the container again filled with blocks of paraffin.

By this method paraffin is slowly dissolved, and as it descends is slowly and uniformly diffused through the xylol, thus preventing, in a large measure, damage to the object by rapid changes in density. It takes longer to reach the saturation point than when solid paraffin is permitted to fall to the bottom of the container, but little plasmolysis results.

Using delicate liverworts for test objects it was found that no deformation of tissue took place, except that which can be accounted for by the excessively large coefficient of expansion (0.00027854) of paraffin. The deformation caused by paraffin in an artificial cell was found to be exactly the same as is always present in plant cells when the paraffin is quickly cooled.

Many workers use a very close series of alcohols in dehydrating and a similarly close series in replacing alcohol with the paraffin solvent, and then undo all their careful work by indiscriminately adding paraffin to the solvent.

A method of fixing paraffin ribbons to the side with certainty

Albumen fixative, which is almost universally used to fasten paraffin ribbons to the slide, has many excellences and a few disadvantages. Among the latter is the property of coagulating when subjected to moderate heat, and in consequence losing its adhesive quality. Because of this it is sometimes impossible to use sufficient heat to straighten refractory or much wrinkled ribbons, especially if paraffin melting at 58 60° is used. Again, it is almost impossible to fix sections of certain refractory plants to the slide, even if the ribbons are first straightened by floating on warm water and then transferred to an albumen coated slide and allowed to dry without heating. This is particularly true of sections of antheridial and archegonial heads of some mosses and of the strobili of *Selaginella*.

In anatomical work with seedlings and sporelings it is necessary to have an absolutely unbroken series, extending sometimes over many slides, in which the loss of a single section would destroy the value of the entire series.

The well known principle that most colloidal substances, when treated with a solution of some salt of chromium, exposed to light and dried, become insoluble in water, was utilized with complete success. The modern photographic processes, such as printing in pigmented gums and gelatin, photogravure, etc., are based on this property of bichromated colloids.

In the Hull Botanical Laboratory the writer and his students first tried Le Page's liquid glue thinned to the consistency of albumen fixative and made slightly yellow by dissolving a small quantity of potassium bichromate in the thinned glue. The slide was smeared with a thin coating of the bichromated glue and dried in the light. Later a solution of gum arabic was tried with even better results.

The present practice is to spread a few drops of a 1 per cent solution of gum arabic on the slide, taking care to see that every portion of the surface is covered, and flood the slide with water made slightly yellow by dissolving in it a few crystals of potassium bichromate. The ribbons are then straightened out on the slide by means of heat, the excess solution drained off, and the preparation put aside in the light to dry. A very short exposure to light is sufficient to render the gum insoluble in water. After the slides are thoroughly dry they are treated in the usual manner.

In heating the slide to straighten out the ribbons no special precaution, such as necessary with albumen fixative, need be taken, since gum arabic does not lose its adhesive power at temperatures below the melting point of the hardest paraffin ever used in imbedding. The paraffin in the ribbon may even be melted without lessening in the slightest the adhesive property of gum arabic.

When a large number of slides are to be made it is very convenient to mix the gum arabic and the potassium bichromate solutions and flood the slide with the mixture. The solutions should be mixed immediately before using, since the mixture does not keep.

Albumen fixative is much improved if, instead of water, the bichromate solution is used.

In the foregoing process the strength of the bichromate solution seems to be immaterial. If it is stronger than 1 per cent, crystals will appear when the preparation dries. These crystals do no harm, since they never appear in the sections, but they leave unsightly spots on the glass. In practice a 0.2 per cent bichromate solution will be entirely satisfactory. The writer does not make a solution of definite proportions, but adds enough potassium bichromate crystals to make the water pale yellow. A very small quantity of a salt of chromium is sufficient, in the presence of light, to render gum and gelatin insoluble in water.

Imbedding in gelatin

In preparing hard woods for sectioning it is the custom to soften in hydrofluoric acid and imbed in celloidin. Since this process involves dehydration, some refractory woods become unmanageable when sectioning is attempted.

In connection with the work of M. A. BRANNON on the extremely hard stems of plants which had been submerged by the rising waters of the Salton Sea and then exposed when the water receded, a method of imbedding in gelatin was devised by the writer and successfully used. Many of these stems were decorticated, some partially macerated, and all were excessively hard.

Gelatin is soaked in water until no more is taken up, the excess water drained off, and the gelatin liquefied by heat. Pieces of wood previously softened in water, or if necessary in hydrofluoric acid, are placed in the melted gelatin for some hours. Small blocks of hard wood to serve as supports in the microtome are also placed in the melted gelatin. The blocks to be sectioned are properly oriented in a gelatin matrix on the supporting blocks, cooled to set the gelatin, and plunged into strong formalin to harden the gelatin. In cutting the knife is flooded with water.

The advantages of this method are that no dehydration is necessary; that the process is very rapid; and that partly disintegrated tissues are held in place. In careful hands sections of hard woods can be cut as thin as is possible by the celloidin process.

Softening refractory material imbedded in paraffin

Plant material, especially if much starch is present, will not cut readily in paraffin. For complete infiltration with paraffin dehydration must be thorough, and a corresponding hardening of tissues results.

As is well known, paraffin is pervious to water. If imbedded material impossible to cut without fragmentation or tearing of the sections be stored in water for some weeks or months it will in most instances section readily. The effect of water on imbedded material will be most strikingly shown if an attempt is made to cut the gametophyte of some of the cycads at or just after fertilization of the egg immediately after imbedding, and again after the paraffin cakes have lain for some months in water. Dormant embryos of *Helianthus* which will not ribbon immediately after imbedding give unbroken ribbons after the paraffin block has been soaked for some weeks in water. The writer stores in water all paraffin containing hard material.

A method of cleaning cover glass

In attempting to clean cover glasses 50 60 mm. long by wiping with a cloth after they have been freed from the cleaning fluid, many are broken even with the most skilful and practiced handling. Also it is almost impossible to have them free from lint.

In the writer's practice cover glasses are placed in the usual cleaning fluid used for laboratory glassware, a mixture of sulphuric acid and potassium bichromate, rinsed under a tap to completely remove the acid, placed while wet in alcohol, and finally completely submerged in 95 per cent alcohol until wanted. To use, the cover glass is slowly withdrawn from the alcohol so that a minimum film of alcohol will remain on the glass, one end touched to a piece of absorbent paper free from dust to remove the drop of alcohol, touched to a flame, and when the alcohol has completely burned off placed while warm on the slide.

This method is very rapid and gives beautifully clean *cover glasses* with practically no breakage. If the cover glass is drawn from the alcohol so slowly that a very thin film remains, a small crack in the cover glass will not spread.

UNIVERSITY OF CHICAGO

A STUDY OF DELAYED GERMINATION IN ECONOMIC SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 204

(WITH ONE FIGURE)

DEAN H. ROSE

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A STUDY OF DELAYED GERMINATION IN ECONOMIC SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 204

DEAN H. ROSE

(WITH ONE FIGURE)

This paper presents the results of an attempt to discover some of the practical problems that seedsmen and growers have to meet, and to work out, so far as possible, practical methods of solving these problems. The seeds tested were furnished by six of the leading seed houses of the United States.

In the present state of our knowledge it can be said that delayed germination and poor germination are due to one or more of the following causes: hard-coatedness, the need of after-ripening, exclusion of oxygen by the seed coat, the effect of frost on seeds, fungi on or in seeds, and of course the presence of seeds containing dead embryos.

Hard-coatedness

The condition of hard-coatedness in the seeds of legumes is well known. To overcome this condition investigators have used hot water, chemicals, and mechanical devices for scratching or puncturing the seed coat.

The use of hot water for forcing germination is undoubtedly older than the references to it in periodical literature. It was recommended by BRUYNING (**6**) in 1893 for seeds of *Ulex europaeus*,

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and by WERNICKE (**30**) in 1895 for several different kinds of seeds. Mention may also be made of the work of JARZYMOWSKI (**17**) in 1905 with seeds of various economic legumes, and of BOLLEY (**3**) in 1912 with those of alfalfa. BOLLEY obtained positive improvement in germination if exposure to a given temperature was not long enough to kill the embryo.

Treatment with concentrated sulphuric acid dates from the work of ROSTRUP (**25**) in 1896 1897. It was also used by TODARO (**29**) in 1901, by HILTNER (**14**) in 1902, by JARZYMOWSKI (**17**) in 1905, and by BOLLEY (**3**), and LOVE and LEIGHTY (**22**) eight years later. Increased germination was obtained in all these cases.

Treatment with other chemicals has included the use of ether, ethyl, and other alcohols (VERSCHAFFELT **31**, 1912), chloroform, sodium hydroxide, postassium hydroxide, potassium nitrate, and mercuric chloride. Of these, the lower alcohols are the only ones that are very effective.

There are obvious practical objections, however, to the use of either hot water or chemicals. As a consequence, there have been numerous efforts to devise means for the mechanical treatment of hard-coated seeds. In Germany, KUNTZE and HUSS (**16**), working about 1890, were able with a scratching machine to increase the germination of *Lathyrus sylvestris* 83 per cent, *Vicia Cracca* 71 per cent, and *Astragallus Glycyphyllos* 77 per cent.

Somewhat later MICHALOWSKI devised an apparatus in which the seeds were passed between two rollers, one of rubber, the other of rough steel. Smaller sorts of seeds were badly crushed by such a mechanism, and it was later displaced by two others, one designed by the Wissinger Seed Co., of Berlin, the other, called a "preparator," by H. NILSSON of the experiment station at Svalöf, Sweden. In both of these the seeds are thrown from a revolving disk against the concave surface of a circular rough stone, within which the disk revolves. HUME and GARVER (**15**), using the "preparator," obtained a definite increase in the germination of seeds of *Medicago sativa*, *M. media*, and *M. falcata*. Another machine now in use in England has made it possible, according to CARRUTHERS (**7**), the designer, to buy clover seed guaranteed to germinate 98–100 per cent. The seeds to be treated are fed into a revolving cylinder lined with sharp, close-set steel points against which the seeds are thrown and scratched as the cylinder revolves.

Mention should be made here also of an apparatus invented by Kühle (**19**) for scraping the rough outer covering from sugar beet "seed." Very satisfactory results have been obtained by its use, since "seeds" so treated absorb water better than untreated ones, and germinate more rapidly; they also give a better total germination, on account of the removal of fungus-infected material from the outside of the "seed," especially if this removal is followed by treatment with some fungicide.

With any one of the machines here described except the last, which serves a slightly different purpose, it has been found difficult to treat every seed that passes through and, at the same time, to avoid serious cracking of the coat or bruising of the entire seed (Glockentoeger **11**).

It is believed that these difficulties have been avoided in a machine devised and in use during the winter of 1912–1913 at the Hull Botanical Laboratory of the University of Chicago. This machine consists of a direct pressure blower, furnished by the Connersville Blower Co., to which is attached an apparatus through which seeds can be fed and blown against the points of a bank of needles. In experiments conducted with this machine, the blower was driven by a two horse-power motor and gave pressures as high as 2.5 pounds to the square inch. The needles used were of three sizes, nos. 4 and 11 sewing needles and no. 4 darning needles, made up into three different cylindrical bunches or banks, each bank of course consisting of only one size of needles. The needles were held together by solder at the eye end and by wire or a ferrule one-half to two-thirds of the distance from the eye to the point.

In the cut here shown (fig. 1) the needles are about half an inch from the end of the air tube. In practice a screen cap is placed over the needles and the tube as a covering for a glass jar beneath, into which the seeds fall. To use the apparatus, valve *e* is closed and valve *b* is opened; seeds are poured into compartment *c*; valve *b* is closed and the blower started; valve *e* is then opened wide enough to let the seeds out, but not so wide that they interfere with each other as they strike the needle points. It is plain that the distance the valve is to be opened will vary with different seeds, but will not be at any time particularly hard to determine. With valve *b* closed, there was no difficulty in getting the seeds down into the air tube; with it open they would be blown out at *d*.

The opening at *h* is five-eighths of an inch in diameter and will accommodate seeds of sweet peas, lupines, *Lathyrus*, honey locust, etc. When smaller seeds are to be treated, an attachment with three-eighths inch feed, valves, and air tube can be adjusted readily. Valve *g* can be used to regulate the pressure, as read on the pressure gauge *f*.

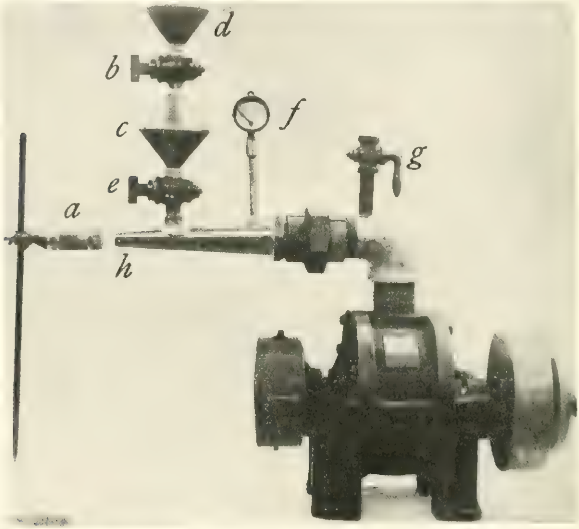

FIG. 1.—Apparatus for increasing the germination of hard-coated seeds by blowing them against a bank of needle points.

Tests were made of treated and untreated seeds, on filter paper kept moist with distilled water, at a temperature of 23-25° C. The results are summarized in table I.

It is plain that treatment with the machine increased germination considerably in the case not only of legumes, but also of snapdragon, *Delphinium*, sweet marjoram, *Ipomoea*, okra, and lettuce.

TABLE I

Germination of seed treated with machine

Kind of seed	Duration of test in days	Germination	
		Untreated	Treated
Alfalfa	10	51	99
Alfalfa	10	74	94
Bossiaea heterophylla	30	3	73
Bossiaea scolopendria	30	11	52
Clianthus Dampieri	10	0	80
White clover	10	70	94
Delphinium chinense	30	74	96
Dilliwynia ericifolia	30	2	77
Gleditschia	90	0	70
Ipomoea (average of 3 spp.)	10	34	71
Lathyrus (average of 3 vars.)	14	63	93
Lettuce (average of 11 vars.)	12	55	83
Sweet marjoram	30	54	96
Mustard (average of 2 vars.)	14	20	69
Okra	30	34	79
Perennial peas (average of 2 vars.)	10	62	87
Sweet peas (average of 5 vars.)	14	70	99
Platylobium trilobatum	30	13	64
Snapdragon	30	6	23
Vetch	10	77	92
Average		40.7	83.7
Increase due to treatment			43.7

It is not strictly correct, however, to call lettuce seeds hard-coated. That their germination is improved by treatment with the machine shows delay to be due to coat restrictions. The coat restrictions are removed also by soaking in water for 24 hours, hence it is likely that delay is caused by a reduction in rate of water absorption rather than by lack of oxygen. Whatever be the character of the seed coat, or coats, which interferes with germination, it disappears as the seed grows older. These points are well illustrated by table II. The data here presented support the statement made by various seedsmen, that two or three-year-old lettuce seed gives better germination than fresh. How this condition comes about is not known, but it probably depends on changes in the permeability of the seed coat. It is certainly not a matter of embryo changes.

TABLE II

GERMINATION OF TREATED AND UNTREATED LETTUCE SEED

VARIETY	DURATION OF TEST IN DAYS	GERMINATION		
		Untreated	Treated with machine	Soaked in H_2O 24 hrs.
Black-seeded butter—				
1909	12	98	.....	.....
1910	12	99	.....	.....
1911	12	89	93	95
1912	12	67	97	100
Prize head—				
1909	12	98	.....	.....
1910	12	97	.....	.....
1911	12	84	96	97
1912	12	70	98	96

It appears, from a study of the records kept in this work, that not only the total germination, but also the rapidity or energy of germination is greater in treated than in untreated seeds. This is shown in table III.

TABLE III

RAPIDITY OF GERMINATION OF TREATED AND UNTREATED SEEDS*

Kind of seed	Germination after	Untreated	Treated
Alfalfa	3 days	48	98
White clover	3 "	69	89
Perennial peas	4 "	0	33
Perennial peas	4 "	24	80
Lupines	4 "	40	88
Delphinium	5 "	4	44
Sweet peas	4 "	0	70
Sweet peas	4 "	16	86

* For final germination percentage of these samples see table I.

Such rapidity of germination would clearly be of advantage in making the crop uniform in size and age, and in keeping down weeds. Indeed, it may be said truly that vigor of germination and vigor of the plants produced are more important than merely high germination percentage. Plants that get a good strong start are more certain to be productive than those that for any reason are weak from the beginning.

Before this machine can become commercially practicable, experiments must be conducted to determine: (1) the possibility of substituting something else for needle points; (2) the proper distance these points should be from each other to give the best results for different sized seeds; (3) the pressure necessary to give the best germination for different kinds of seeds; for certain legumes a pressure of two to three pounds is necessary, for lettuce one pound or even less; (4) the effect of storage on the germination of treated seeds; (5) the germination of treated seeds in soil. To be effective in overcoming hard-coatedness, the needle point need only pass through the palisade layer and not entirely through the coat. Even with this slight deformation it is possible that bacteria and fungi can gain an entrance. That destruction by bacteria and fungi actually does take place was shown by JARZYMOWSKI (**17**) for seeds of *Ulex europaea*, lupines, and other large-seeded legumes which had been treated with the Wissinger machine. Red clover and *Lotus corniculatus* were the only ones whose germination in soil after treatment was not seriously reduced.

As to the germination in soil of seeds treated by the blowing method here described, there are not at present enough data on hand to justify the drawing of definite conclusions. Preliminary experiments seem to indicate for alfalfa seed, where the percentage of hard seed is high, that germination in soil is definitely better after treatment than before. Further investigation, of course, is necessary before this can be confirmed. In conclusion it may be stated that there was no serious crushing or cracking of seeds or seed coats by this machine.

The need of after-ripening

This is a condition which occurs, to mention only a few cases, in seeds of *Crataegus*, various conifers, *Fraxinus*, potato tubers, and lily-of-the-valley bulbs. For a discussion of the general situation and a résumé of the literature the reader is referred to the paper by ECKERSON (**10**) dealing with after-ripening in the seeds of *Crataegus*. The work to be discussed here had to do with the germination of seeds of conifers, and specific reference will be made to a few of the more pertinent papers on the subject.

It is a matter of common knowledge that conifer seeds germinate slowly. It is also well known for several of them that as they grow older the rapidity of germination increases, up to at least the end of the first 6 months after they were gathered. SCHWAPPACH (**27**) states that in the fall seeds of *Abies* did not begin to show sprouts for 60 days, and required 40 days more before the test could be considered closed. In March they began almost immediately and finished in 20 days. The conclusion is natural that after-ripening takes place, and this, in fact, is assumed by workers who have recently attacked the problem. HILTNER and KINZEL (**14**), it is true, reasoning from results obtained by treating seeds of *Pinus Strobus*, *P. Peuce*, and *P. Cembra* with concentrated sulphuric acid, ascribed the delay to coat restrictions. LAKON (**20**) has made the objection that the tests on which these authors rely were too few and on too small a number of seeds. He repeated their experiments with the same three species of pine, but could obtain no forcing of germination. Untreated seeds took up water just as well as did the treated ones, even though their outer coats were hard. Careful determinations of the amount of water absorbed by untreated seeds of *Pinus sylvestris*, *P. Strobus*, *P. Peuce*, and *P. Cembra* showed that all of them reached nearly the maximum in 24-48 hours. Increases in weight after that time were practically negligible; hence it is clear that such seeds cannot be considered "hard-coated" like the seeds of legumes. Moreover, the cutting test, applied to these seeds, showed that all of them were damp, that is, had absorbed water. Increases in weight, therefore, were not due to a few easily swelling seeds. From these results LAKON concludes that conifer seeds are not, strictly speaking, "hard-coated," and that delay is due to conditions within the embryo.

Although LAKON found concentrated sulphuric acid ineffective, CORREVON states, in a paper published somewhat earlier, that weak acid (0.25 per cent acetic or 2 per cent phosphoric) increases the germination of seeds of *Juniperus Cedrus*.

SCHWAPPACH recommends cold storage for 14–30 days (he does not say how cold) for seeds of *Pinus Strobus*, followed by a germination temperature of 25° C. The common practice of layering various conifer seeds doubtless finds its justification in a shortening of the time required for germination. Low temperatures have also been used for preserving the vitality of conifer seeds. HAACK (**12**) dried seeds so they lost about 2 per cent in weight, then stored them in dry, air-tight containers on ice. CLEMENS (**9**) stored seeds in the refrigerator of a brewery in vessels containing sodium carbonate to absorb moisture and carbonic acid. Both investigators report that seeds thus stored remained viable longer than those kept under ordinary laboratory conditions.

In order to analyze the situation more carefully, the following series of experiments were conducted on seeds of various conifers.

1. Tests of untreated seeds.

2. Tests of seeds which had been in cold storage (3–5° C.): (*a*) in wet sand, (*b*) in weak solutions of hydrochloric acid, (*c*) in distilled water.

3. Tests were made with seeds which had been injected with weak hydrochloric acid or with water, by exhausting the air from them when they were in these liquids, and then restoring the pressure to normal. This was repeated at least three times for all seeds here spoken of as injected. Table IV summarizes the results obtained in series 1 and 2*a*.

TABLE IV

GERMINATION OF CONIFER SEEDS, UNTREATED AND AFTER STORAGE IN WET SAND AT 3–5° C.; PERCENTAGE GERMINATING AFTER ONE MONTH IN GERMINATOR

KIND OF SEED	DRY STORAGE UNTREATED				COLD WET STORAGE FOR			
					1 month	2 months	3 months	4 months
	Date of starting germination							
	Jan 26	April 4	April 24	May 30	Feb. 26	Mar. 25	April 26	May 28
Cupressus macrocarpa . . .	8	0	1	—	15	27	40	36
Picea Menziesii	56	42	.	23	70	60	.	. .
Pinus austriaca	57	48	27	28	78	88	.	64
Pinus Strobus	12	10	6	28	34	40	44	59

The results given in the table show that germination was definitely increased by cold wet storage for four kinds of conifers.

In no case does the maximum germination of seeds of a given kind from dry storage equal that of seeds of the same kind from cold wet storage. The effect of the cold wet storage is most noticeable in the cases of *Pinus Strobus* and *Cupressus macrocarpa*, where increases of 32 per cent and 31 per cent respectively were obtained. For all of the seeds here reported on, except those of *Pinus Strobus*, dry storage seemed to cause a decrease in viability. This can be seen from the first four columns of the table. Tests of *Pinus Strobus* were run for 60 days, the other three for 30 days.

TABLE V

GERMINATION OF SEEDS OF CONIFERS AFTER SOAKING IN WEAK ACID AND STORAGE AT 3–5° C.

KIND OF SEED AND LENGTH OF TIME IN COLD STORAGE	CONTROL	ACID (HCl)					WATER	
		Soaked			Injected			
		n/20,000	n/10,000	n/6,400	n/20,000	n/6,400	Soaked	Injected
Pinus Strobus—								
3 days	.	44	58	53	66	54	. .	.....
6 "	.	51	57	44	. .	. .	. .	.....
10 "	.	53	65	67	. .	. .	63	.....
No cold storage	30	.	. .	.	. .	. . .	50	68
Pinus austriaca—								
3 days	.	64	.	52		.	65	....
6 "	. .	48	. . .	42	. .	. . .	59	.....
10 "	.	35	.	50	.		45	.....
No cold storage	52	54	. .	. .	. .	. .	.....	.....

The series of experiments shown in Table V was planned to determine whether delay in germination is due to an alkaline or neutral reaction of the embryo. It was thought that if such is the case, weak acid solutions would change the reaction sufficiently to cause growth to begin, when the seeds were placed in the proper conditions.

The results obtained for *Pinus Strobus* do not, however, bear out this theory. Seeds injected with distilled water gave 18 per cent better germination than those merely soaked in it, and slightly better than those injected with weak hydrochloric acid. It seems likely from this that delay is due merely to lack of water. When this water was supplied, by long soaking or by forcible injection under pressure, germination was much improved.

Results for *Pinus austriaca* are less conclusive on this point. They do show, however, and the same is true for *P. Strobus*, that soaking in either water or weak acid gave greater germination than was obtained in the controls, 38 per cent and 13 per cent respectively. For *P. austriaca*, better results were obtained from short than from long soaking.

Referring again to table IV, it is possible that the increases in germination shown there were due not so much to the cold storage in itself as to the thorough infiltration of the seeds with water. There is need of much more work on this question before any definite conclusions can be drawn.

Exclusion of oxygen

No attempt will be made to review former work, since this has already been done by SHULL (**28**). Results presented in table VI seem to indicate that the germination of certain economic seeds is delayed for lack of oxygen. They also indicate the need of a detailed study of these seeds.

TABLE VI

GERMINATION OF SEEDS TREATED WITH OXYGEN OR HYDROGEN PEROXIDE

Kind of seed	Duration of test in days	Untreated	In 80 per cent oxygen	In 0.15 per cent H_2O_2
Dandelion	14	50		72
Datura—				
Golden Queen	14	73		70
Wrightii	14	20		100
Lettuce—				
Grand Rapids	10	0	44	32
Martynia	20	0	90	80

Datura Wrightii was forced considerably by hydrogen peroxide, *Datura* Golden Queen not at all. Lettuce gave good results, but, as has been suggested, this is probably due to absorption of water.

Effect of frost on seeds

ATTERBERG (**2**) says that seeds of oats and barley harvested in Sweden after a heavy frost gave fair to good germination in the laboratory, but in many cases no plants when sown in the field. Unpublished data obtained by EASTHAM in Canada show that the germination of oats grown in the prairie provinces is often seriously reduced by early frosts. He says, "as far as our observations go, a couple of degrees of frost in the milk stage are in many instances sufficient to ruin oats for seed. In the dough stage they are not nearly so susceptible, and when well ripened and dry stand considerable frost without serious injury." EASTHAM found also that such seed, germinating poorly when harvested, often improved with age. This seems to indicate in such cases the necessity for a period of after-ripening. Through the courtesy of the Canadian seed laboratory and two American seed houses the author has had the privilege of testing several samples of frosted oats. The results are summarized in table VII.

TABLE VII

GERMINATION OF OATS

VARIETY	UNTREATED; ON FILTER PAPER AT 20° C.	UNTREATED; SENDER'S TEST 18°–20° C.	HULLS OFF; ON FILTER PAPER AT 20° C.	HULLS REMOVED; ON COTTON IN PETRI DISHES AT 22° C.			
				80 per cent oxygen	60 per cent oxygen	40 per cent oxygen	20 per cent oxygen
Lincoln	85	40	79	......	......	......	......
Swedish	77	40	80	......	......	......	......
4920....	34	35	38	31	25	38	33
5139....	46	......	......	......	......	......	......
3477....	56	43	......	......	......	......	......
3974....	45	42	......	......	......	......	......
4948....	89	62	94	93	97	95	96
5302....	16	27	......	......	......	......	......

The best germination was obtained from hulled seeds in oxygen, though the results are more clear-cut for no. 4948 than for no. 4920. No definite conclusions can be drawn as to what percentage of oxygen is most effective. It is noteworthy that two samples, nos. 3477 and 4948, show much better germination, 23 and 27 per cent respectively, than they did when tested in the Canadian Seed Laboratory six months earlier. This agrees well with the statement made above that frosted oats go through a process of after-ripening and improve in viability as they grow older. There is the same need of after-ripening in wild oats (*Avena fatua*), as has been shown by Atwood. But referring to the table again, it will be seen that one sample, no. 5302, deteriorated in vigor as it grew older, since it gave a percentage of 27 when first tested and 11 per cent less 6 months afterward. Considering the results as a whole, it is clear that frosted oats are unreliable in performance and of very doubtful value for seeding purposes.

Another crop which sometimes suffers from frost is garden peas. Within the last two or three years the growing of garden peas for seed has become an important industry in Idaho and Montana. It has been found that certain of the late varieties grown there are injured by frost and the viability of the seed seriously impaired.

A study of 14 samples of such peas has shown that decrease in germination is probably due to two different causes, both of which, however, may be the effect of frost.

1. Actual injury to the embryo, especially the tip of the radicle. It has a whitish shriveled appearance and starts to grow very slowly, if at all.

TABLE VIII

Germination of peas

Variety	Coats on; 20°	Coats on; 25°	Coats off; 20°
Premium Gem........	90	82	80
Nott's Excelsior 73186..	68		
" " 71531	98	100	
Telephone 885H.......	58	46	55
Gradus 913K.........	44	30	58
" 913S.	70		76
Dwarf Defiance 874C..	56		52
" " 874K...	64		96
" " 874H..	78		76
Alderman 912S........	66		
" 912K...	82		96
" 912T....	56		
" 912V .	78		92
Telegraph 68528.......	48		50
Average..........	67		74

2. The presence of fungi on or in the seed coat. That this actually decreases germination was shown by the work summarized in table VIII. With the coats on, the seeds of all the samples here reported on showed much fungus infection; with coats off,

very little or even none at all. Ten samples gave an average germination of 67 per cent with the coats on, and 74 per cent with the coats off, a difference of 7 per cent. Individual samples, such as Dwarf Defiance 874K, gave even more striking results.

The condition of garden peas, with reference to fungi, is approached more or less closely by that of a large number of other garden and flower seeds as is shown in table IX, summarizing the general results of this investigation, and in the discussion following.

Plants whose seeds show delayed germination, classified according to probable causes (the word "probable" is used advisedly, for while the evidence is convincing in some cases, it is much less so in others):

1. Hard-coatedness.—*Canna*, *Clianthus Dampieri*, *Delphinium*, *Erythrina*, *Hibiscus*, *Ipomoea* (4 spp.), *Lathryus*, *Lupinus*, sweet peas (4 vars.), snapdragon, alfalfa, sweet clover, white clover, lettuce (10 vars.), mustard (2 vars.), okra, sensitive plant, sweet marjoram, vetch, *Gleditschia*.

2. Frosted.—Oats, peas (8 vars.).

3. Need of after-ripening.—Wild cucumber, *Picea* (3 spp.), *Pinus* (2 spp.).

4. Exclusion of oxygen by the seed coat.—*Datura Wrightii*, *Martynia*.

5. Cause of delay not determined.—*Coix Lachryma*, feather grass, Pampas grass, asparagus, barley, blue grass, cardoon, celery, chives, dill, horehound, kaffir corn, leek, millet, parsley, parsnip, pepper, radish, rosemary, spinach, summer savory, thyme, *Aquilegia*, *Asparagus Sprengeri*, *Bignonia*, *Centaurea*, *Clematis*, dandelion, *Datura* Golden Queen, *Eschscholzia*, foxglove, heliotrope, *Helianthus*, hop, lavender, *Momordica*, *Nasturtium*, *Oenothera*, pansy, *Pentstemon*, *Primula*, *Salvia*, *Verbena*, *Abies Mertensiana*, *A. pectinata*, *Berberis*, *Betula alba*, *Cupressus horizontalis*, *C. macrocarpa*, *C. pyramidalis*, *Larix*. Further work would doubtless explain the cause of delay in many of these seeds and make the growing of plants from them a much simpler matter than it now is.

Very pertinent at this point are the results from the Minnesota Seed Laboratory for 1910 and 1911 (OSWALD **23**). Of field seeds 14 kinds were tested; of garden seeds 26 kinds. Of field seeds, for

TABLE IX

SHOWING THE NUMBER OF SPECIES AND VARIETIES TESTED, CLASSIFIED ACCORDING TO THE QUALITY OF GERMINATION AND THE PROBABLE CAUSES OF DELAY

SPECIES AND VARIETIES	GERMINATION				PROBABLE CAUSE OF DELAY						INFECTED WITH FUNGI
	Good	Poor or slow	None	Totals	Hard-coated-ness	Frost injury	Exclusion of oxygen by seed coat	Need of after-ripening	Not de-termined	Totals	
Ornamental grasses			3	3					3	3	
Field and garden plants.....	22	44	2	68	16	9			19	44	31
Ornamental flowering plants . .	11	35	3	40	19		2	1	21	43	26
Shrubs and trees	..	9	5	14	1			5	8	14	12
Totals........	33	88	13	134	36	9	2	6	51	104	69

3 of the kinds, 50 per cent or more of the samples were below the government standard of germination. Of garden seeds, for 16 of the 26 kinds, 50 per cent or more of the samples were below standard. Mention should be made also of work by BROWN (**5**) on the germination of packeted vegetable seeds. He found that the average germination of box vegetable seeds put up by 60 firms for four years was 60.5 per cent. The lowest average for any firm was 36.5 per cent, the highest 81.5 per cent. The average germination of packeted vegetable seeds put up by 20 mail-order houses in 1911 was 77.5 per cent (lowest average 76.2 per cent, highest 77.5 per cent). Just what these figures signify is not clear. There are three possibilities: (1) the seeds were poor because of the seedsman's dishonesty or carelessness; (2) the seeds were poor because it is not possible with present methods to produce better ones; if so, the government standard is, now at least, too high and methods of production need improvement; (3) the seeds seemed poor because present methods of making germination tests do not always adequately determine the value of a given sample. In the writer's opinion, the responsibility for low test must be shared about equally by all three, though the first is a less important factor than it was a few years ago.

6. Plants whose seeds were found infected with fungi.—Feather grass, asparagus, beggar weed, buckwheat, cardoon, celery, chives, sweet clover, dill, kaffir corn, leek, millet, oats, parsley, peas (12 vars.), pepper, radish, rosemary, spinach, thyme, vetch, *Aquilegia*, *Asparagus Sprengeri*, *Bignonia*, *Clematis*, *Clianthus Dampieri*, wild cucumber, dandelion, *Datura* Golden Queen, *D. Wrightii*, *Helianthus*, hop, *Ipomoea* (4 spp.), *Lathyrus*, lavender, *Nasturtium*, pansy, sweet peas (4 vars.), *Primula*, *Verbena*, *Abies Mertensiana*, *A. pectinata*, *Berberis*, *Cupressus horizontalis*, *C. macrocarpa*, *C. pyramidalis*, *Picea excelsa*, *P. Menziesii*, *P. rubra*, *Pinus austriaca*, *P. Strobus*.

The species and varieties tested were 134, but 30 of these are omitted from the second section of table IX; 29 of these germinated rapidly and well, and one other, on account of bad infection with fungi, showed not delayed but definitely poor germination; 69, or 51.4 per cent of the total, were found more or less infected with fungi. This point was determined for each kind of seed not more than two days after the test began. All filter paper was boiled 5–10 minutes before being used and kept moist with distilled water during the test. Repeated washing of seeds and removal to fresh filter paper showed that in all cases infection came from the seeds, not from the paper.

There is no intention here of implying that seedsmen in general purposely put on the market seeds low in vitality or badly infected with fungi. It does seem clear, however, that there is need of closer supervision by the seedsmen themselves of all stages of the process of seed production; alternation of crops to avoid soil-infection, cultivation, harvesting, threshing, cleaning, storage; all of these need close attention if seed of the best quality is to be produced. The most candid way in which to approach the whole question is to admit that seed analysts, seed-growers, and seed merchants do not at present know a number of things they need to know in reference to the question of fungus infection of seeds, and to all the other questions considered in this paper. The whole matter constitutes an extremely complex physiological and pathological problem, with very practical aspects, the solution of which can be brought about only by careful study from several different points of view. To be specific, the following lines for investigation may be suggested:

1. The relation of germinator tests to the actual vegetation of seeds in the soil. This should be studied through a period of several years.

2. The relation of fungi on or inside of seeds to the germination of such seeds in soil. At the risk of seeming to repeat unnecessarily, the writer wishes to say that in his opinion the importance of this problem is only poorly appreciated in this country. Some recognition of the dangers accompanying fungus infection of seed has appeared of late in the work of BOLLEY and others in the United States, and in German agricultural literature. APPEL (1), writing on the relation of pathology to seed control, says that in seed-testing stations, pains should be taken to give judgment as to the presence of spores of plant diseases on seeds to be examined. It is his opinion, further, that in comparative field tests more

attention must be given than formerly to pathological phenomena. Observations on this point should be given along with other data from the experiment.

3. The causes of delayed germination in asters, certain hardy perennials, labiates, ornamental grasses, cucurbits, conifers, frosted oats, *Betula*, and *Berberis*.

4. The value of hard seeds of legumes when planted in the soil.

5. The relation of any or all of the causes of delayed germination to the vigor of the plants produced. It is not enough that a given lot of seeds shall be free from impurities; it is not even enough that it shall give a high germination percentage. It must, above all, give rise to vigorous productive plants, when planted in field conditions. Consequently, any knowledge which will teach us how to grow such seeds and how to know poor seeds is of the greatest practical importance.

Summary

1. Hard-coated seeds of legumes, and seeds of *Delphinium*, *Ipomoea*, lettuce, mustard, okra, sweet marjoram, and snapdragon can be forced to more rapid germination by being blown against needle points.

2. For two varieties of lettuce it is shown that the seed improves in viability as it grows older, up to the end of at least the fourth year. This improvement is probably due to increased permeability of the inner seed coat to water.

3. Cold storage in wet sand increased the germination seeds of *Pinus Strobus* by 32 per cent, of *Cupressus macrocarpa* by 31 per cent. Delayed germination of conifer seeds, more especially those of *Pinus Strobus* and *P. austriaca*, seems to be due to lack of water intake, and not to an alkaline or neutral reaction of the embryo. This statement is supported by the fact that seeds injected with distilled water gave better germination than those merely soaked in water or in weak acid at the temperature of melting ice. Any kind of soaking or injection gave 13–38 per cent better germination than was obtained with the controls.

4. Certain samples of frosted oats improve in germinating power as they grow older, others deteriorate.

5. Certain late varieties of western-grown garden peas germinate poorly. This is shown to be due to one or both of two causes: (*a*) actual frost injury to the embryo; (*b*) the presence of fungi on or in the seed coat or inside of it.

6. Seeds of 51.4 per cent of all species and varieties examined showed fungi on the seed coat within two days after being put to germinate.

The writer is indebted to Dr. William Crocker for many suggestions and criticisms.

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THE EFFECTS OF ILLUMINATING GAS ON ROOT SYSTEMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 205

(WITH NINE FIGURES)

EDWARD M. HARVEY AND R. CATLIN ROSE

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Introduction

The injurious effects of illuminating gas upon trees and shrubs have been testified to by numerous observers. In 1864 GIRARDIN (5) reported severe injuries to trees in several cities of Germany which he attributed to escaping illuminating gas. Similar observations have since been recorded by many writers, among whom are VIRCHOW (17), KNY (8), SPÄTH and MEYER (14), EULENBERG (4), WEHMER (18), SHONNARD (13), and others.

The shade tree commissions of every city find themselves face to face with this serious problem. The trees of our city streets and parks are unusually subject to the various tree-injuring agencies, the chief of which are insects, fungi, and atmospheric and soil impurities. The two latter factors are particularly characteristic of the troubles of city trees. The problem of gas injuries, therefore, is one of considerable economic importance. City foresters should know the exact cause of any tree death, not only to enable them to provide means for future protection, but also in order to determine with whom responsibility rests for the present financial losses. They should therefore be able to say with certainty whether or not a tree has been killed by gas in the soil. At present this is no small undertaking, because there are few, if any, reliable symptoms known by which one may differentiate with certainty gas injuries from those due to several other causes. For example, fungi sometimes quickly become prominent in a tree injured by gas, as purely a secondary effect (see KNY 8 and STONE 15); but in a case like this the primary injury might easily be attributed to the fungi. It is claimed that characteristic odors often accompany gas poisonings; sometimes in the roots as well as in the soil; in fact, these odors form the chief diagnostic characters. STONE (15) claims much for them, and it is probably true that in many cases they furnish some evidence. However, many instances are known where illuminating gas odors have not given sufficient evidence for fixing responsibility. A rational system of diagnosis of injuries in city trees would be of value and would be welcomed by all parties concerned. No such system, from the gas injury standpoint, is possible, owing to the lack of definite knowledge concerning the effects of the constituents of illuminating gas under various controlled conditions.

The experimental work previously done on gas injuries to root systems will be briefly summarized. KNY (8) passed known amounts of illuminating gas through the soil at the roots of a maple and two linden trees. Among the symptoms of injuries recorded were the bleaching and final fall of leaves and the appearance of a blue coloration in the xylem of the roots. Finally the trees died. SPÄTH and MAYER (14) passed small amounts of gas into soil in which grew a number of species of trees and shrubs. General death resulted, but otherwise the only symptom recorded was the yellowing of the leaves. BÖHM (1) grew willow slips in water into which he had passed a stream of illuminating gas. The short roots produced soon died, but the slips themselves lived for three months. Potted plants of *Fuchsia* and *Salvia* died after gas had flowed to their roots for four months. Again, he found that when gas had been passed through a soil for a long period of time, this soil became very toxic to seedlings germinated in it. Also a *Dracaena* transplanted to this soil became dry and died in ten days. MOLISCH (10) found that roots of corn increased in diameter and were bent in certain concentrations of illuminating gas. SHONNARD (13) noted exudation of sap from the trunk and branches of a lemon tree treated with gas in the soil. RICHARDS and MACDOUGAL (11) found that carbon monoxide and illuminating gas retarded the rate of elongation of roots of *Vicia Faba*, sunflower, wheat, rice, etc. Swelling also appeared in the leaf sheaths of wheat, being somewhat more pronounced in illuminating gas than in carbon monoxide. Such increases in thickness were largely due to the enlargement of the cortical cells. In some cases, however, the cambium seemed to have become more active. Recently STONE (**16**) has reported proliferations of tissue at the lenticals of willow slips growing in water which had been charged with illuminating gas. He also noted a rapid proliferation at the cambium in stems of *Populus deltoides* due to the influence of gas.

Another important phase of the gas injury problem is that after trees have been killed by gas, a question arises regarding the safety of resetting trees where the dead ones have been removed, assuming, of course, that the gas leak has been located and stopped. It seems to be the general opinion that resetting should only be done either after a considerable time has elapsed, or after large amounts of the old soil have been removed and replaced by fresh soil. Neither of these methods of procedure is entirely satisfactory; the first involves great loss of time, the second is expensive. The practicability of resetting trees in any given case is often determined only by the crude method of smelling a handful of soil taken from the place of injury, and if the odor of gas is still present, resetting is deemed unsafe. One is thus led to ask whether the odor itself is a true index of the toxicity of the soil to the roots of plants.

The investigation reported below was undertaken with the two problems in mind: (1) that of determining some of the effects of illuminating gas on root systems, having in mind the securing of further diagnostic characters of gas poisoning; and (2) whether the chief causes of injury are those constituents of illuminating gas which are readily absorbed by the water film of the soil particles, or those which remain mainly in the soil interstices (not being so readily soluble).

Methods and materials

The illuminating gas used was the so-called "water gas" from the Chicago Gas Light and Coke Company's system. Along with the illuminating gas experiments, many parallel ones were carried out with an ethylene-air mixture. The Chicago illuminating gas contains 2.6 per cent ethylene; therefore, to facilitate comparison between the ethylene alone and the ethylene of the illuminating gas, the ethylene of the mixture above was made to constitute 4 per cent (by volume). Thus, volume for volume, the ethylene-air mixture and the illuminating gas contained approximately the same amount of ethylene. In the following experiments, where the term "ethylene in corresponding concentrations to the illuminating gas" is used, it refers to the ethylene content of the latter gas. For example, the ethylene concentration corresponding to an illuminating gas concentration of one part gas to four parts of air ("1:4") would be one part ethylene to 100 of air ("1:100"). Such parallel experiments were considered important owing to the fact that ethylene has been found to be by far the most toxic constituent of illuminating gas for the aerial organs of several plants (see CROCKER and KNIGHT **2**, KNIGHT and CROCKER **7**, and HARVEY **6**).

The experiments were primarily arranged so as to yield evidence with regard to the two phases of the second problem above. The methods will be described separately and in the order named. Investigation of the second problem should yield data with regard to the first.

A. THE TOXICITY OF THE CONSTITUENT OF ILLUMINATING GAS ABSORBED BY THE SOIL

Good potting soil in 10–20-liter cans was treated with illuminating gas by allowing the gas to flow through at a definite rate, at room temperature, for varying lengths of time. The rate of flow was approximately 2 liters per hour. The time periods were from 30 hours to 20 days, hence the lots of soil received 40–1000 liters of gas. The moisture content of the soil was kept as near the "optimum" as possible. In one experiment 10 liters of soil received gas at the rate of about 0.2 liter per hour for 68 days. In another experiment 8 liters of soil received gas at the rate of 2 liters per hour for 53 days, and was kept at a temperature of 1–5° C. throughout the period. The purpose of this soil treatment was to allow the soil particles to absorb as much of the gas constituents as possible. In the case of treatment at low temperature, it was the intention to allow still better opportunity for condensation of substances on the soil particles. After stopping the flow of gas, the soils were removed from the cans and thoroughly stirred in pure air to free them from the gas in the interstices. They were then taken to the greenhouse, placed in shallow boxes, and planted to 41 different species of plants, representing 18 families. Controls were maintained throughout. The seeds were allowed to germinate and grow for periods of 25–60 days.

B. THE EFFECT OF ILLUMINATING GAS ON ROOTS WITH NO SOIL PRESENT

1. *With roots alone exposed to gas*

In order to expose the roots without exposing the shoots, the following method was employed. Moist-air chambers were made from 8-liter, wide-mouthed bottles; 6–8 short glass tubes were inserted in the corks of each of these bottles so that the tap roots of young seedlings of *Vicia Faba* could be admitted to the chamber. The space between a root and the sides of a tube was sealed by means of a short length of pure gum tubing which had been previously fitted to the outer end of the glass tube. Definite amounts of illuminating gas or ethylene were admitted through a small glass tube reaching to the bottom of the bottle. The pressure resulting from the addition of the small volume of gas was relieved through a second short tube.

2. *With entire plant exposed to gas*

Seeds of tomato, radish, and mustard were allowed to germinate, and when the hypocotyls had reached a length of 0.5 cm. they were transferred to flower pots under bell jars provided with water seals. The seedlings were fixed to the rims outside of the flower pots, so that the roots in growing would hang free in the air. Definite amounts of illuminating gas could be easily added.

C. EFFECT OF ILLUMINATING GAS ON ROOTS GROWING IN SOIL MEDIUM

1. *Quantitative tests*

In these tests young seedlings (two or three months old) of *Catalpa speciosa*, *Ailanthus glandulosa*, and *Gleditschia triacanthos* were used. A few days before the beginning of the experiments, the seedlings were transplanted from the pots, in which they had germinated, to large battery jars filled with coarse quartz sand. Two glass tubes for admitting gas were thrust into the sand and then the roots and glass tubes were sealed in with a vaseline-paraffin mixture by the BRIGGS and SHANTZ method. The total volume of the interstices (allowing for a definite water content) was previously determined, and upon the basis of this volume, known concentrations of gas could be secured. The duration of experiments was 5–21 days. Gases were changed every three days by drawing pure air through the jars for 10–15 minutes by means of an aspirator, and new gas added. Parallel experiments with ethylene were also run in many cases.

2. *Qualitative tests*

In this series of experiments it was desired to subject a number of plants to illuminating gas under conditions met with in the field where leaking gas mains are involved. Potted woody plants, including 36 individuals of 11 species, were used. Illuminating gas was allowed to pass slowly into the soil around the roots. Meanwhile symptoms of injury were noted. When the plant had died, or become seriously injured, or after a certain time had elapsed, the roots were washed clear of soil, and careful examinations made. The rate of gas flow was often less than one-eighth liter per hour.

Results and discussion

The results will be presented in the order in which the methods were described.

A. EFFECT OF ILLUMINATING GAS ON SEEDS PLANTED IN SOIL PREVIOUSLY TREATED WITH GAS AND THEN AERATED

In all cases, at the time of the planting of the seeds, the treated soil gave an exceedingly strong odor of illuminating gas. Of the 41 species planted, 5 failed to germinate, but the failure was in both the controlled and the treated soils. Throughout the 25–60 day period, the aerial portions of the seedlings were watched for signs of injury. However, it was found that the plants in all the treated soils gave no superficial evidence of injury. All seemed perfectly normal. At the end of the period the plants were taken up, their roots washed free of soil, and examinations made. Only two species gave any evidence of injury; these were cotton and lupine. In them the root systems were perhaps somewhat less developed in the treated soils, and in the cotton there appeared to be a greater development of anthocyanin in the treated soils. At the close of the experiments, the treated soils always gave a noticeable gas odor and in most cases the odor was very strong.

The results of these experiments would indicate that of the constituents absorbed from illuminating gas by soil, those which give the odor to the gas are very prominent, and when once absorbed are held for extended periods, even after the soil is freely exposed to the air. But the most important fact from the standpoint of the question in hand seems to be that these odorous constituents are evidently not extremely toxic to plant roots growing in the same soil. The plants tried in such soils included a wide range in regard to relationship, and also they were taken at what is considered the critical stage, that is to say, the germinating and young seedling stages. Just what these odorous compounds are is an interesting question. The odor of any illuminating gas is probably the combined odor of a number of substances, for example, pyridine, thiophene, picoline, quinoline, cumene, cymene, and others. Very little is known concerning the effect of these on vegetation. Crocker, Knight, and Rose (3) found cumene, thiophene, and pyridine were many hundred times less effective in reducing growth in the etiolated sweet pea seedlings than was ethylene.

The results of the experiments described indicate that the presence of a gas odor in soil is not an index of its toxicity for vegetation, and that odors would be valuable merely as a means of determining whether or not illuminating gas had been in the soil. With regard to using odors in diagnosis, Crocker has suggested to us the possibility of distilling (at high temperature *in vacuo*) soil from places where gas injuries have been suspected but where the odor even at the time is not discernible, thereby drawing off some of the odors previously held too firmly by the soil particles.

B. THE EFFECT ON ROOTS WITH NO SOIL MEDIUM

1. *With roots alone exposed*

Material, seedlings of *Vicia Faba*. Exposure period five days.

With illuminating gas. (1) Concentration 1:4000; no effects were noted, evidently grew as well as controls; (2) concentration 1:400; growth in length somewhat retarded and two other strongly marked effects entered: (*a*) swelling back of the root tip, and (*b*) turning and coiling of root (fig. 1); (3) concentration 1:40; growth in length greatly retarded, also considerable swelling; coiling present, but the coils smaller and tighter.

With ethylene.—(1) Concentration 1:100,000 (that is, corresponding to ethylene of illuminating gas with (1) above); apparently no effect; normal growth ensued; (2) concentration 1:10,000; responses similar to those shown in fig. 1 but somewhat more pronounced; (3) concentration 1:1000; little or no growth in length; strongly swollen, no coils.

The parallel experiments with ethylene gave some evidence that the toxic effect recorded for the illuminating gas may be due to ethylene present in that gas because corresponding concentrations of illuminating gas and the ethylene-air mixture gave quite parallel results.

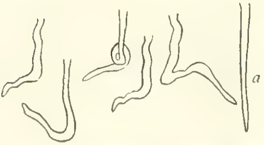

FIG. 1.—Outline of *Vicia Faba* roots, showing the effects of illuminating gas on growth; *A*, control.

2. *With entire plants*

Material, radish, mustard, and tomato. The roots and hypocotyls gave responses as follows:

Radish.—Exposure period 48 hours.

With illuminating gas.—(1) Concentration 1:500; bending of root evident (similar to those of fig. 1); no coiling; no swelling of hypocotyl or root; (2) concentration 1:5000; coiling and bending of root evident; no enlargement of hypocotyl or root.

Mustard.—Exposure period 48 hours.

With illuminating gas.—(1) Concentration 1:100; coiling and bending of root evident (similar to those of fig. 1); swelling of hypocotyl evident; no swelling of root; (2) concentration 1:11,000; bending of root slight; no other effect; (3) concentration 1:20,000; bending of root slight; no other effect.

Tomato.—Exposure period 3 days.

With illuminating gas.—(1) Concentration 1:500; swelling between stem and root (fig. 2); growth considerably retarded; (2) concentration 1:10,000; swelling as above, but less marked;

swollen zone longer and not so thick; more growth of hypocotyl; (3) concentration 1:100,000; little if any effect.

With ethylene.—(1) Concentration 1:12,500; short swollen knob between stem and root; growth greatly retarded; (2) concentration 1:250,000; swollen knob longer and not so thick as in (1); (3) concentration 1:2,500,000; little if any effect.

The response shown by the tomato seedling differs very markedly from that shown by the radish and mustard seedlings. While the roots of the radish and mustard seedlings show a coiling and bending similar to those of the *Vicia Faba*, in addition to a slight swelling of the hypocotyl, the only response shown by the tomato seedling is a decided swelling of the hypocotyl and root at the point where the two join. With the tomato seedling the parallel experiments with ethylene again give some evidence that the toxic effect recorded for illuminating gas is due to the ethylene constituent of that gas.

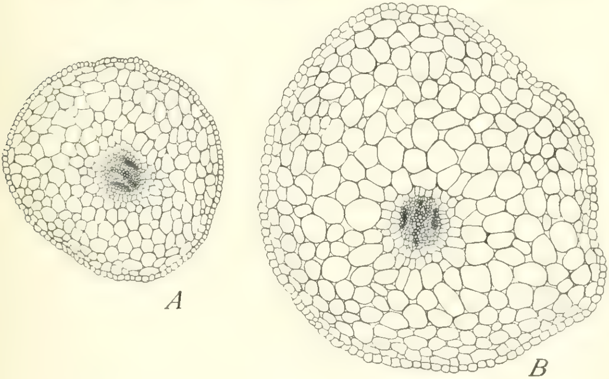

FIG. 2.—Sections of the root of tomato seedlings: *A*, control; *B*, treated with illuminating gas; ×18.

The results recorded above for *Vicia Faba*, radish, and mustard show that injuries to roots may readily be brought about by placing them in an atmosphere containing small amounts of illuminating gas or ethylene. If we consider here also the results of the first series of experiments, it would seem that the chief causes of injury to root systems are those constituents of illuminating gas which are present mainly in the interstices of the soil, rather than those dissolved in the soil water.

The greatest danger in replanting where other plants have been killed by gas, seems to lie in the constituents remaining between the soil particles. If, therefore, a method was devised for quickly aerating this soil, trees might be safely planted at once without removing large amounts of soil.[1]

C. THE EFFECTS OF ILLUMINATING GAS ON ROOTS IN SOIL MEDIUM

1. *Quantitative tests*

a) *Catalpa speciosa* seedlings; illuminating gas; exposure period 8 days; concentrations 1:2000, 1:400, 1:200, 1:40, 1:20, and controls.

Stems and leaves showed no modifications; neither were there any strongly marked effects on the root systems. In concentrations 1:40 several roots gave indications of swelling 1-2 cm. back of the tips, while in concentrations 1:20 these swellings were very evident.

b) Same as above, with the exception of ethylene in place of illuminating gas but in corresponding concentrations (that is, 1:50,000, 1:5000, 1:1000, and 1:500).

No effect on stems or leaves. The responses with concentrations 1:1000 and 1:500 were like those above, but in addition, the tendency of the roots to coil at the tips (as noted in *Vicia Faba*, fig. 1).

c) *Catalpa* seedlings; illuminating gas; duration of exposure 21 days; concentration 1:4 and controls.

Stem and leaves gave no response. Roots of 1:4 gave swelling of main root extending from near the surface of the sand 4-7 cm. downward (fig. 3). This increase in amount was 2-3 times that of the normal thickness. The epidermis was often cracked and sloughed off in places. The drawing of fig. 4 shows that the swelling is brought about through an increase in size of the cortical cells, and particularly by rapid proliferation at the phellogen layer.

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[1] Perhaps this could be accomplished by passing pure air through the soil by means of a pipe thrust below the surface.

d) *Ailanthus* seedlings; illuminating gas; exposure period 15 days; concentrations 1:400, 1:10, and controls.

1:400 gave slight swelling in tap root near the surface of the soil; while in the 1:10 the leaves began falling after 5 days, and had all fallen before the end of the exposure. The tap roots were much swollen for 3–4 cm. below the surface of the soil.

e) Same as (*d*) but with ethylene instead of illuminating gas; concentrations 1:10,000 and 1:250.

Swollen zone of tap root was somewhat more pronounced in the 1:10,000 than in the 1:400 illuminating gas above. In the 1:250 all plants had lost their leaves within 8 days, and the tap roots were much swollen. The morphological nature of these swellings may be seen in figs. 5 and 6. In fig. 6 are two outlines of transverse sections of the tap roots of *Ailanthus* seedlings in the region where swellings take place; *A*, the control plant; *B*, a treated plant. Fig. 5 shows the detailed structures of the regions outlined in *A*

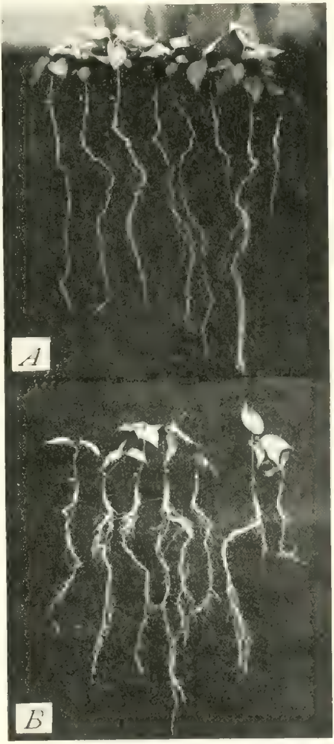

FIG. 3. *Catalpa* seedlings: *A*, controls; *B*, treated with illuminating gas.

and *B* respectively of fig. 6. Through an examination of figs. 5 and 6 it becomes evident that the stelar region has remained unchanged, while the cortex, extending to the phellogen layer, has increased in thickness, partly through increase in the diameter of the cells, and partly through cell division. At the phellogen layer of the treated root, cell division has been rapid, resulting in the production of a loose tissue not present at all in the normal. The tissue lying outside the phellogen layer in the beginning has been only slightly modified in the abnormal root.

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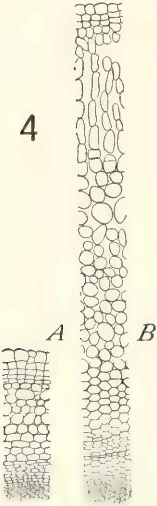

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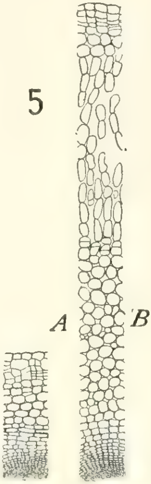


FIGS. 4, 5.—Fig. 4., section through the cortex of tap root of *Catalpa: A*, control; *B*, treated with illuminating gas; fig. 5, same for *Ailanthus* roots, showing detail of the tissue regions outlined in fig. 6; ×48.

f) A number of tests were carried out with *Gleditschia* seedlings, used in illuminating gas in various concentrations up to 1:3 (concentrations higher than 1:3–1:4 were not used for fear of oxygen becoming a limiting factor). Concentration 1:3 gave leaf fall, but no definite injuries were detected in the root system.

2. *Qualitative tests with potted woody plants*

When illuminating gas was passed rapidly through the soil of a potted plant, injuries might be observed the first day and death in all cases in the course of a few days. This is not at all surprising, because one should expect injury and final death to result as an effect of the shutting off of the oxygen supply, even though an inert gas (that is, nitrogen or hydrogen) be passed through the soil in this manner. Accordingly, illuminating gas could do great damage to vegetation independent of any direct toxic property. This view is advanced by KOSAROFF (**9**), who found that wilting took place if a stream of carbon dioxide or of hydrogen was passed through soil in which roots were growing. In all experiments where illuminating gas was passed into the soil rapidly enough to cause the death of the plant, the symptoms manifest in the aerial parts were of a type which would indicate that the injury might be due simply to the cutting off of the water supply as a result of injury to the root system, and not, necessarily, due to a conduction of toxic substances to them. With high evaporating conditions, the symptoms of gas injury (that is, wilting, yellowing, and falling of leaves in the aerial portions) always became manifest very much sooner than under conditions favoring a low water loss. It was found that when roots were killed quickly by using high concentrations of gas, few if any symptoms other than odors appeared which might enable one to diagnose the death as specifically gas poisoning. With regard to the injury to parts above soil, STONE (**16**) believes that "poisonous principles" are absorbed by the roots and conducted upward to the leaves, hence the yellowing and wilting would result as a direct poisoning of them.

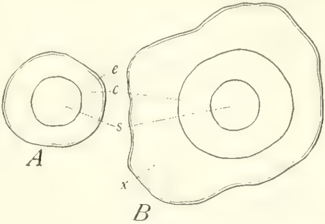


FIG. 6.—Diagrams of transverse sections of *Ailanthus* roots; *A*, control; *B*, treated with illuminating gas; *s*, stele; *c*, cortex; *e*, epidermis and tissue lying outside of phellogen; *x*, new tissue developed by the phellogen; ×6.

When illuminating gas was so regulated that it passed very slowly to the roots (sometimes as little as 40 cc. per day), a stimulation of the roots often took place, resulting in the development of new tissue resembling those reported from the quantitative experiments made with young tree seedlings. Stems were likewise affected when covered by soil or when slightly above the surface.

In stems this proliferation of cells became first evident at the lenticels by their increase in size and the protruding of white tissue, as shown in the lower portion of the stem in fig. 7. RICHTER (**12**) has noted a similar proliferation of tissue at the lenticels of *Vicia villosa* under the influence of tobacco smoke. STONE's similar results have been cited. In the roots this tissue was abundantly developed in some cases, particularly in *Hibiscus*, which is shown in fig. 7, where *B* and *C* are plants which were subjected to a slow stream of illuminating gas for a period of 30 days. Fig. 8 shows detailed structure of these proliferations in the roots of *Hibiscus*. The cork layer of the abnormal root has been sloughed off. Practically the same morphological situation appears in this case as in *Ailanthus* previously figured.

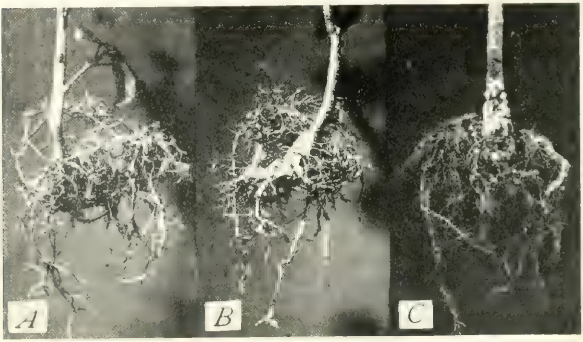
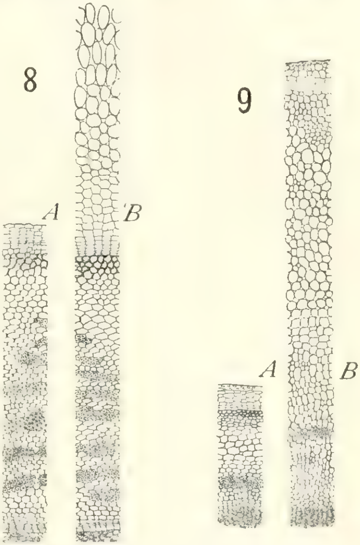


FIG. 7.—*Hibiscus:* *A*, control; *B*, *C*, treated with illuminating gas

A similar response by lilac is shown by drawings of fig. 9. These abnormalities were recorded for *Hibiscus*, lilac, *Croton*, *Diervilla*, *Ricinus*, *Ulmus*, and pear.

An experiment similar to the foregoing was carried out with an *Ailanthus* growing on the campus near the Hull Botanical Laboratory. The tree had a diameter of about 8 cm. and a height of 3.5 m. At a short distance were other trees of the same species which served as controls. Illuminating gas was admitted to the roots of the experimental tree through a glass tube thrust 0.7 m. into the soil, 0.6 m. from the base of the tree. The rate of flow of gas was 1.5 liters per hour. The experiment began July 3, and the gas was stopped flowing September 2. Except for two or three short periods, amounting in all to less than three days, the flow of gas was continuous during the two months. Thus the soil near the tree must have received 1.5–2 cubic meters of gas.

The first symptoms of injury were manifest July 14. Leaves of some of the young shoots, growing on the same side of the tree from which the gas entered the soil, showed signs of wilting. Three days later these leaves and others had shriveled and died, but remained attached to the shoots. In some cases only a portion of a leaf was injured. A few leaves of older branches also wilted, but there was no general effect evident throughout the tree at that time. In the middle of September the apparently unaffected leaves began to fall, and finally the tree was free from leaves much before those of the controls. In October the tree looked as though it were entirely dead, and when an examination was made, after it had been removed from the soil, such was found to be the case. A general dryness of the tissues was noted, but neither anatomical changes nor gas odors were detected in them.



FIGS. 8, 9.—Fig. 8, transverse sections through cortex of *Hibiscus* at base of stem; ×40; *A*, control; *B*, treated with illuminating gas; fig. 9, same for lilac; ×35.

During the gas flow, the odor of gas was evident only at the point where the tube entered the soil. In order to determine whether the gas might be detected by a more delicate means, the etiolated sweet pea seedling test, as described by KNIGHT and CROCKER (7) was tried. When the seedlings were 1.5–2 cm. high, the petri dishes containing the seedlings were taken to the *Ailanthus* tree, placed directly on the soil, and the 10-liter cans inverted over them. The cans, each with a dish of seedlings beneath, were numbered and placed, with reference to distance from the point of entrance of gas, as follows: no. 1, 5 cm., toward tree; no. 2, 0.6 m., at base of tree; no. 3, 0.8 m., also near tree but on side opposite no. 2; no. 4, 1.2 m., on side directly opposite point of entrance of gas; no. 5, 3 m., as no. 4. The cans were placed August 27 and observations were made three days later. The results were as follows: no. 1 gave no growth (observations refer to epicotyls only); no. 2, some growth, with a little swelling; no. 3, growth slightly reduced and diageotropic; no. 4, slender and straight, 9–10 cm. high (normal); no. 5, as no. 4. These results indicate that the pea seedling probably offers a rather delicate test for the presence of illuminating gas in the soil. In this case the injurious effect on the seedlings was very evident in those placed near the base of the tree, where, as stated before, no odor of gas could be detected in the customary manner.

In consideration of the great difference in behavior of a given plant when exposed to low and to high concentrations of illuminating gas, it seems appropriate to make the following suggestions with regard to the diagnosis of gas injuries. One reason why the foregoing abnormal tissue developments have not been recorded in trees killed by gas is probably the fact that examination was made for pathological symptoms in trees which have died or have become seriously injured. Such conditions would mean that the gas had been at their roots in too high concentration to allow the stimulating effects to enter. Sometimes the proliferation reported above was found in roots when no suggestion of injury could be observed in the aerial portions. Therefore, when one is attempting to diagnose with certainty a serious injury suspected to be due to gas, he ought also to make an examination of roots of other trees in the vicinity which have not yet shown injuries in the leaves, thereby perhaps adding one more line of evidence. Another change found to take place under the influence of low concentrations of illuminating gas was the disappearance of starch from the cortex of the roots, an observation in agreement with that of Richter (12) and others.

Summary

1. When illuminating gas is passed through soil, the odor-giving constituents of the gas are readily absorbed by the soil particles and strongly held.

2. These odorous substances are very slightly, if at all, toxic to roots of plants growing in a soil containing them.

3. The constituents of illuminating gas which remain in a gaseous state in the soil interstices are the chief cause of injury to root systems.

4. Among these constituents, ethylene is probably the most harmful, except in extremely high concentrations of illuminating gas, where the toxicity of other substances, together with other factors, would be expected to play a part.

5. Low concentrations of gas induce abnormal development of tissue.

a) Illuminating gas.—These abnormalities appear in certain tree seedlings within 8–21 days, with concentration one part illuminating gas to four parts air (air of the soil), or as low as one part illuminating gas to forty of air.

b) Ethylene.—This gas alone, when used in concentrations corresponding to the ethylene content of the illuminating gas used in the tests, gives abnormalities similar in type and degree.

6. High concentrations of illuminating gas result in the rapid killing of the roots, and the only symptom of injury to be observed is death.

7. If illuminating gas is allowed to flow very slowly through a soil in which woody plants are growing, abnormal tissue development in the root will very often ensue.

8. In low concentrations of illuminating gas, hydrolysis of starch and some other related chemical reactions are accelerated.

9. It was found that, by use of the etiolated sweet pea seedling, small amounts of illuminating gas in the soil could be detected where the odor of gas was indistinguishable by the usual methods.

We wish to acknowledge our indebtedness to Dr. William Crocker of this laboratory for many valuable suggestions during the course of this investigation.

University of Chicago

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GAMETOPHYTE OF PELLIA EPIPHYLLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 206

(WITH PLATES I-IV AND ONE FIGURE)

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GAMETOPHYTE OF PELLIA EPIPHYLLA

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A. H. Hutchinson

(WITH PLATES I–IV AND ONE FIGURE)

Three of the species of *Pellia—P. epiphylla*, *P. calycina*, and *P. endivaefolia*—show morphological differences, especially with respect to the apical cell, which would suggest a generalized or possibly an unstable ancestral form. No detailed study of any of these species has been reported. During the investigation upon which this account is based, it has been found that in *P. epiphylla* not only are there transitions in the method of growth, but also that the development of the antheridium may follow any one of several divergent lines.

Antheridium

It has been generally accepted that "the antheridium of *Pellia* is larger than that of *Aneura*, but its development is very similar, except that the stalk is multicellular, as it is in other *Anacrogyneae*."[1] In addition to this method of development, *P. epiphylla* shows young antheridia having characters of Marchantiales; moreover, the spermatogenous initials may be cut out in the same way as the primary axial cell of the archegonium.

The division of a dorsal cell, the third or fourth from the apical cell, by a horizontal cross-wall is the first evidence of an antheridial initial. The outer of the two cells formed divides again, giving the three cells of the antheridial row—the basal cell, the stalk initial, and the outer cell; the latter by successive divisions gives rise to the wall cells and spermatogenous cells. Meanwhile, the dorsal cells, immediately surrounding the antheridial group, divide and become papillate, thereby producing a ring-shaped involucre (figs. 2, 3). The outer cell of the antheridial group divides next. The position of the wall is significant; if it is vertical and median, the successive divisions follow an antheridial sequence; if, however, the wall is inclined and somewhat removed from the central position, the cell divisions which follow are similar to those of a developing archegonium. In the former case the vertical wall is followed by a curved wall on either side, which cuts the vertical wall as shown in figs. 7 and 8. Two similar walls, rotated about the central axis through an angle of 90° (figs. 8, 9), complete the separation of the peripheral region from the central spermatogenous region. The first two of these walls may be nearly parallel at the base (fig. 7), or they may be at right angles; similarly the second pair. Such an antheridium is characteristic of the Jungermanniales.

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[1] Campbell, D. H., Mosses and ferns. New York. 1905 (p. 92).

Occasionally the outer cell, mentioned above, is divided into quadrants by walls at right angles to the vertical wall (figs. 10, 11), in which case four wall cells are cut off by periclinal divisions, giving also four spermatogenous cells. The process is similar to that characteristic of *Sphaerocarpus* or Marchantiales.

Fig. 14 illustrates the result of a combination of these two methods of development. In fig. 15 is shown a double antheridium; the two halves have become completely separated by the vertical division and each has developed independently. The process may be compared to the characteristic development of the double antheridia of Anthocerotales.

When the first wall formed in the outer cell is inclined and somewhat removed from the median position, it is followed by a second and a third wall, each of which is similarly placed, but revolved with respect to each other through an angle of 120° about the vertical axis (figs. 17, 18, 26, 27, 28). A transverse wall divides the central cell into the cap cell and the spermatogenous initial (fig. 19). The characteristic archegonial development is followed until the massive spermatogenous group begins to be formed (figs. 22, 28), instead of the axial row.

Occasionally this critical third wall of the antheridium is inclined inward instead of outward, as described above (fig. 23). Two walls similarly inclined complete the separation of the peripheral region from the central spermatogenous initial (figs. 24, 25). This form is similar to that last described with the exception of the inclination of the walls and the resulting lack of the cap cell.

The differences in these antheridia are emphasized by the fact that in the first form (fig. 8) there are two spermatogenous initials, paired as in Jungermanniales; in the second form there are four, arranged in the form of a quadrant (fig. 10), as in Marchantiales; and in the third and fourth there is but one spermatogenous initial (figs. 10, 24, 25).

That the structures described above are developing antheridia, and not archegonia, is evidenced by their position and by the presence of an individual involucre. The antheridia are single and dorsal, while the archegonia are grouped in the terminal pocket.

Archegonium

The position of the archegonial group is of considerable morphological importance. CAMPBELL, with reference to the work of JANCZEWSKI, states: "The archegonia are formed in groups just back of the apex but he [JANCZEWSKI] does not seem to have been able to detect any relation between them and the apical cell such as obtains in *Aneura*, but it is possible that such a relation does exist." As mentioned above, the archegonia are terminal and inclosed in a "pocket," which is formed by a cup-shaped involucral growth. As will be described more fully, the archegonia arise from an apical group of cells, any of which may become an archegonium initial. There is no regular succession in the formation of archegonia; apparently old and young organs are indiscriminately intermingled. Since the apical group ceases to function as such after the production of archegonia, *P. epiphylla* may be regarded as truly acrogynous. The involucre is produced by cells which are cut off laterally by the apical group (fig. 40), and pushed out very much as the wings during the previous period of growth; in this event, however, the lateral cells are forced out on all sides to form a complete inclosure.

The structure and development of the archegonium conforms, in general, to the characteristic liverwort form. Some specific characters may be noted.[2] "After the archegonial mother cell is cut off it does not divide at once by vertical walls, but a pedicel is first cut off [fig. 31]; after which the upper cell undergoes the usual divisions." This character serves to emphasize the similarity which exists between the archegonium as shown (figs. 31–33), and the antheridium (figs. 16–21). Except for the position and presence or absence of the involucre, these organs would be difficult to differentiate until the spermatogenous group or the archegonial axial row, as the case may be, begins to develop. The cap cell gives rise to a group consisting of more than the usual number of cells. The first division of the cap is often simultaneous with the division which gives rise to the primary neck canal cell and the primary ventral cell (figs. 32, 35), and four cap cells may appear in cross-section when there are but three neck canal cells and a ventral cell (fig. 36). Fig. 38 shows a group of cap cells, 6 in cross-section, which may be compared with those which form the neck of the archegonium of Filicineae. JANCZEWSKI reports that the number of neck canal cells may be as high as 16 or even 18; 9 is the greatest number seen by the writer (fig. 38). The venter becomes massive before fertilization; it may be 2 or 3 cells in thickness; a many-celled stalk is also formed (fig. 37). In the young archegonium the neck has usually only 5 vertical rows of cells. The cells originating from the third wall cell do not divide until the archegonium approaches maturity (fig. 39).

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[2] CAMPBELL, D. H., Mosses and ferns. New York. 1905 (p. 90).

Methods of growth

A certain form of apical cell may usually be given as characteristic of a genus or even of a larger group. In *Pellia*, however, there is no such conformity; the apical cell of *P. calycina* has four cutting faces, two lateral, a dorsal, and a ventral—the cuneate apical cell. The dolabrate apical cell of *P. endivaefolia* has but two cylindro-convex cutting faces; while that reported as characteristic of *P. epiphylla*, the lenticular cylindric apical cell, has a posterior convex and two lateral cutting faces. In the last named species, however, there are several methods of growth; these cannot be sharply delimited, but for clearness five rather distinct forms may be taken as characteristic of successive periods of growth.

During the time of intra-capsular gametophytic division a massive body is formed. There is no regional growth, but all cells have an equal power of division. This period of growth is of short duration. The gametophyte body retains this massive form until after the resting period.

The second period of growth begins by the formation of a cuneate apical cell. A terminal cell is cleft by a wall inclined about 30° with reference to the longitudinal axis of the spore; a second inclined wall bisects the first at an angle of approximately 90°; lateral walls complete the cuneate apical cell (fig. 41). The latter cuts off dorsal and ventral segments (fig. 42) as well as lateral segments (fig. 43). Such a method of growth is characteristic of the young gametophyte until about the time of antheridium formation.

The growth of the third period is by means of a lenticular cylindric apical cell (fig. 47). The transition is somewhat irregular. Sometimes the apical cell is more or less equally divided; then the two halves simultaneously cut off cells (fig. 45) which correspond to the dorsal and ventral segments of the preceding form. The thallus in cross-section has the appearance of being medianally divided by a wall. Another transition form is shown in fig. 44. As the thallus becomes thicker the posterior angle becomes greater, the two faces being finally replaced by one which is curved. The cells cut off from the posterior face divide rapidly; as many as 6 segments may be formed before the next division occurs. The rapid division of the laterally placed cells causes the wings to be protruded outward and forward (figs. 46, 48, 49). There has been much discussion regarding the method of branching. HOFMEISTER[3] believed that the central papilla ("Mittellappen") shown in fig. 14 was the seat of the chief apex; hence that there is no true dichotomy. LEITGEB[4] states that the origin of the central papilla is from a marginal cell. When branching takes place, the apical cell, instead of cutting off a lateral segment, divides equally or almost so; each of these daughter cells assumes apical characters. The central papilla is produced by the crowding together of lateral segments from the two apical cells. It later develops into a central lobe corresponding to fused wings. Branching is essentially dichotomous; although the thallus may appear to have a central axis along the main line of growth, it is a matter of comparative rapidity in growth rather than origin. This form of apical cell is continued throughout the antheridial period.

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[3] HOFMEISTER, W., Higher Cryptogamia. Ray Society. 1862.

[4] LEITGEB, H., Untersuchungen über die Lebermoose. 1882. Vols. II and III. Jungermannieen.

The fourth period of growth is terminal and regional; it is concerned with the production of the archegonial pocket. The cells surrounding the original apical cell assume the power of cutting off lateral and posterior segments. The region of growth is in the form of a terminal disk. Lateral segments are crowded out on all sides to produce the continuous, cup-shaped involucre. A cross-section in any plane is similar to a horizontal section through the growing region at the time of branching (fig. 49). This growth is checked by the production of archegonia; any of the surface cells of the pocket may produce an archegonium (fig. 40). About the time of fertilization the last period of growth begins.

Any further growth is in connection with the developing sporophyte. Starch accumulates in the cells surrounding the foot and a massive growth takes place. Usually only one sporophyte develops in each pocket; a rather thick calyptra is formed about it, and sterile archegonia are carried along; these are to be seen attached to the surface of the calyptra. The first growth of the gametophyte is massive, similarly the last.

Relation of antheridium and archegonium

The relationship of the various forms of antheridia and their relation to the archegonium is demonstrated by the occurrence of antheridia in a single species, *Pellia epiphylla*, which are similar in development to each of these organs. The Marchantiales condition is generally to be found among the first antheridia; the Jungermanniales form is most dominant at the middle period, occurring, however, throughout the complete antheridial period. The archegonial form is usually found among the last antheridia to be produced. There is an evident time relation between these forms. Moreover, one may be regarded as derived from another through a series of progressive sterilizations (text fig. 1).[5] In the form characteristic of Marchantiales (figs. *A*, *B*, *C*), the vertical median wall (1) is followed by two walls (2 and 3) at right angles to the former, thereby forming quadrants (*A*, *B*, *C*, *D*). Four periclinal walls (4, 5, 6, 7) form a sterile wall cell and a spermatogenous cell

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[5] Cf. HOFMEISTER, The higher Cryptogamia. Untersuchungen über die Lebermoose. Vol. II (*Pellia*).

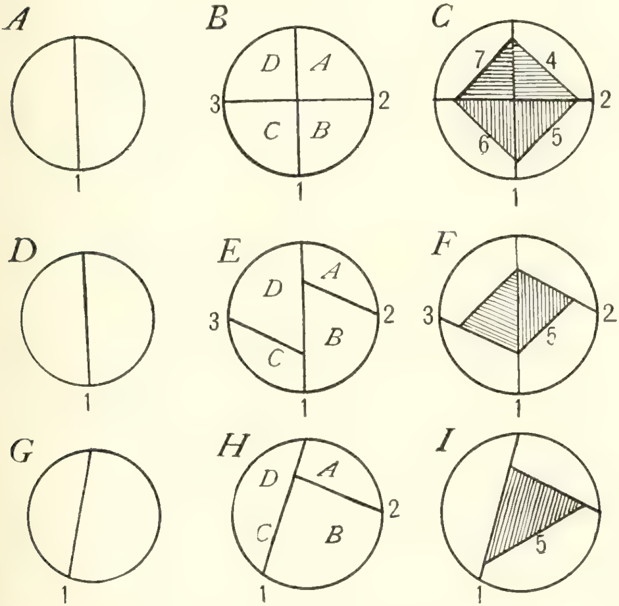

FIG. 1.—Explanation in text

from each quadrant. In the Jungermanniales form (figs. *D*, *E*, *F*) the median vertical wall is followed by two walls corresponding to the second and third above, but somewhat inclined (2, 3, fig. *E*). Periclinal walls (5, 7) form two centrally placed spermatogenous cells. The quadrants *A* and *C* have become sterile wall cells; only the quadrants *B* and *D* give rise to spermatogenous cells. In the archegonial form (*G*, *H*, *I*) the first wall (1) is not median, but somewhat laterally placed and inclined; there is no wall no. 3; the section corresponding to the quadrants *C* and *D* does not divide, but remains as a sterile wall cell. Wall 2 again cuts off a section corresponding to quadrant *A*, which also persists as a sterile wall cell. A periclinal wall (5) divides the section corresponding to quadrant *B* into a wall cell and a spermatogenous cell. Three of the quadrants have become sterile. Starting with the Marchantiales form of antheridia, by the sterilization of alternate quadrants the Jungermanniales form is derived; and by the sterilization of three quadrants the archegonial form results. The sterilization sequence corresponds to the time sequence as described above.

Only one step is lacking in this series to complete the transition from the antheridium of Marchantiales to the archegonium, namely the reduction of the spermatogenous mass into a single row of cells, only one of which, the egg, shall be functional. Such transition forms have been described by DAVIS[6] in Marchantiales. The writer has seen such "archegonia" with gamete masses in sections prepared by Dr. W. J. G. LAND. *The antheridial forms of P. epiphylla furnish evidence that the various types of antheridia and the archegonium have had a common origin*, possibly gametangia resembling in structure the antheridium of Marchantiales.

Relationships

The genetic relationships of *P. epiphylla*, because of its diversity, present a rather complex problem. With respect to its relation to other species of the genus *Pellia*, if the other species retain throughout life the form of apical cell ascribed to them, it would follow that *P. epiphylla* has branched sooner from the general line of progress and has retained and developed generalized characters. As a member of the Jungermanniales, this species is acrogynous in the sense that growth is checked, apical growth is stopped, by the production of archegonia. It differs from the more characteristic Acrogynae in having several regions of growth, each of which is checked in the same way. It is possible that acrogyny has been reached by several lines of development; hence close relationship with "Acrogynae" is not necessarily implied. If the development of the antheridium may be taken as a basis of classification, it would seem that *Pellia* arose from the main line of advance before the two branches, Jungermanniales and Marchantiales, became separate, even before the archegonium and antheridium became definitely differentiated in their methods of development.

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[6] DAVIS, B. M., The origin of the archegonium. Ann. Botany **17**: 477–492. 1903.

From *Pellia* we have evidence regarding the relation of Jungermanniales and Marchantiales. There is in the life history a transition from the cuneate apical cell of Marchantiales form to the lenticular cylindrical apical cell found in certain Jungermanniales; similarly, the antheridium, already discussed, affords strong evidence for the existence of such a relation. The evidence tends to indicate that Marchantiales are primitive and that Jungermanniales are derived.

Summary

The antheridium.—The development varies. The dominant method is that characteristic of Jungermanniales; forms occur, not infrequently, which are like the antheridium of Marchantiales, while others are like the archegonium in their early development.

The archegonium.—The archegonia are produced from cells of the apical group and occur in an archegonial pocket. The diversities from the regular form are few; the large number of neck canal cells, the extreme development of the cap, the frequent reduction of the number of tiers of neck wall cells to five, and the somewhat massive venter may be noted. The outer cell of the two resulting from the division of the archegonial initial divides horizontally before the vertical wall is formed.

Methods of growth.—Several periods of growth may be recognized, each having a specific method of growth: the massive; the period of the cuneate apical cell extending until antheridium formation; the period of the lenticular cylindric apical cell, or the antheridial period; the period of regional apical growth, or the period of archegonium production; and the second period of massive growth, or the period of sporophyte dependence.

I am greatly indebted to Professor JOHN M. COULTER for suggestions and criticisms received, and to Dr. W. J. G. LAND, under whose direction the work was done.

UNIVERSITY OF CHICAGO

EXPLANATION OF PLATES I–IV

All drawings were made with the aid of the Abbé camera lucida. Original magnification of figs. 15, 40, and 50 was 500; of all other figures 1730. The reduction in reproduction is one-third.

FIGS. 1–29.—The antheridium of *Pellia epiphylla*.

FIGS. 1–6.—Longitudinal sections of a young antheridium, such as is characteristic of Jungermanniales.

FIGS. 7–9.—Cross-sections of the same.

FIGS. 10–12.—Cross-sections of antheridia of *Pellia* similar to those of *Sphaerocarpus* or Marchantiales.

FIGS. 13, 14.—Sections through the stalk (fig. 13) and spermatogenous region (fig. 14), the latter illustrating two methods of growth.

FIG. 15.—A double antheridium.

FIGS. 16–22.—Longitudinal sections of antheridia showing a development similar to that of an archegonium.

FIGS. 23–25.—Similar to last, with the elimination of the cap cell.

FIGS. 26–29.—Cross-sections of the same.

FIGS. 30–40.—The archegonium.

FIGS. 30–38.—Longitudinal sections.

FIG. 39.—Cross-section.

FIG. 40.—Cross-section of archegonial pocket.

FIGS. 41–50.—Growth structures.

FIG. 41.—A cuneate apical cell in young gametophyte which has just escaped from the spore coat.

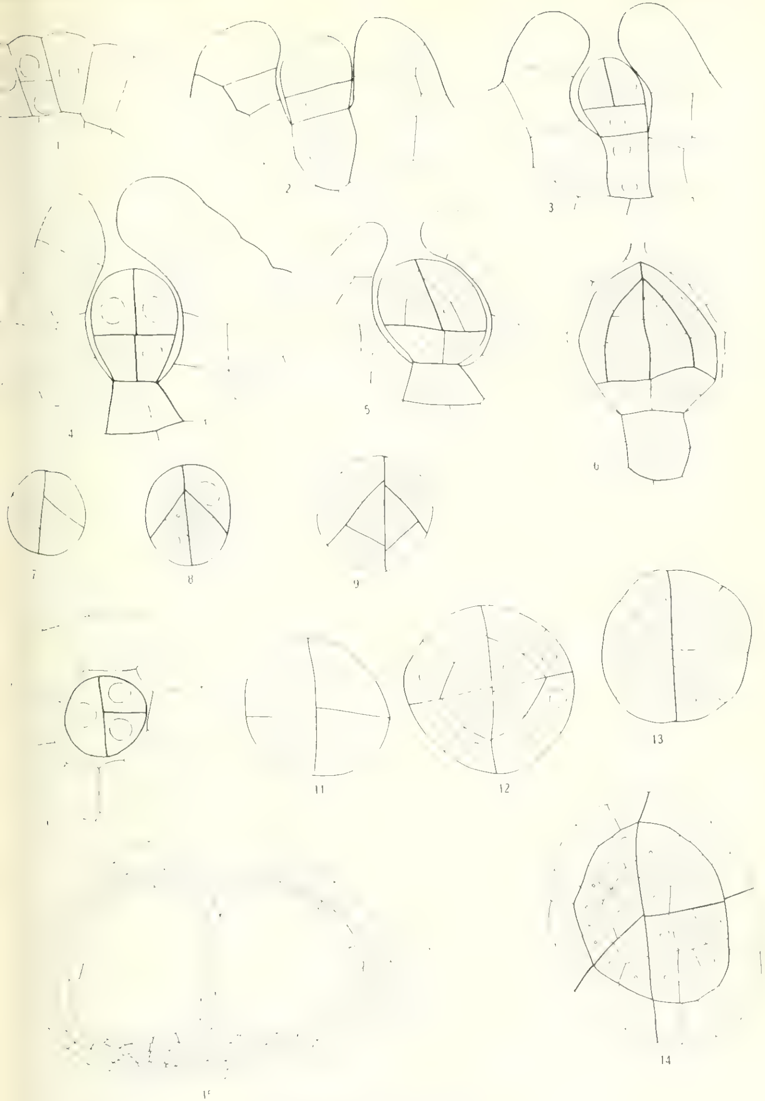
FIGS. 42, 43.—Sections of sporeling showing the segmentation of the cuneate apical cell; fig. 42, vertical; fig. 43, horizontal section.

FIGS. 44, 45.—Transition forms of apical cells.

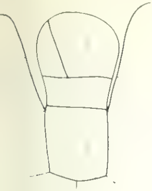
FIGS. 46–48.—Sections of the lenticular cylindric apical cell, also showing the region surrounding; fig. 46, a transverse vertical section; fig. 47, a longitudinal vertical section; fig. 48, a marginal longitudinal vertical section, showing how the lateral segments produce the wing or lobe.

FIG. 49.—A section through a region of apical growth.

FIG. 50.—A young sporophyte inclosed by a somewhat massive calyptra and the involucre; sterile archegonia on calyptra.



HUTCHINSON on PELLIA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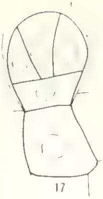
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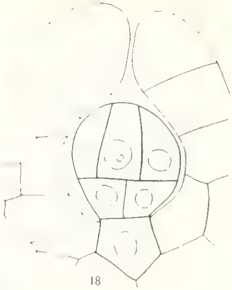
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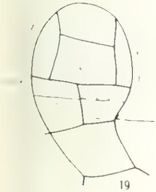
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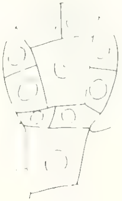
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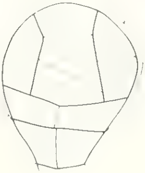
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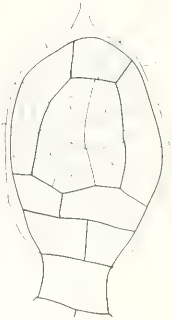
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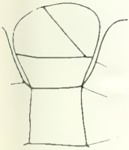
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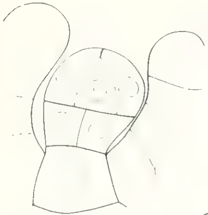
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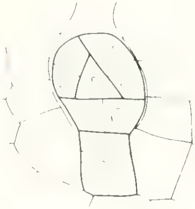
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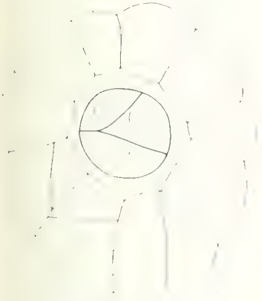
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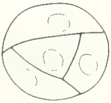
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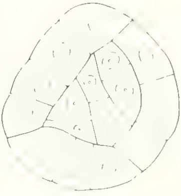
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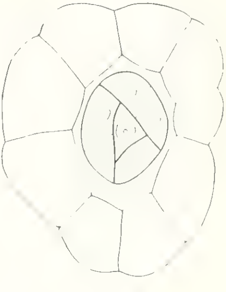
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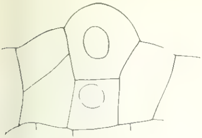
HUTCHINSON on PELLIA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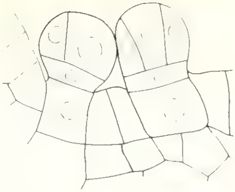
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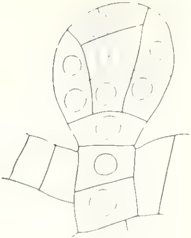
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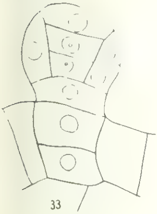
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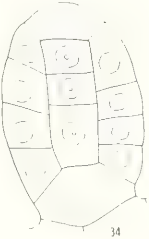
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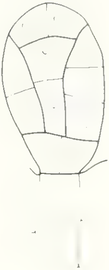
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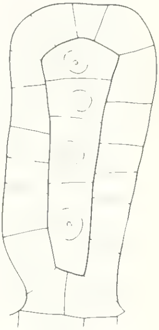
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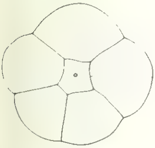
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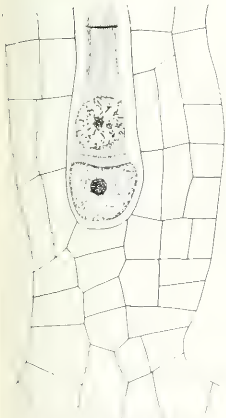
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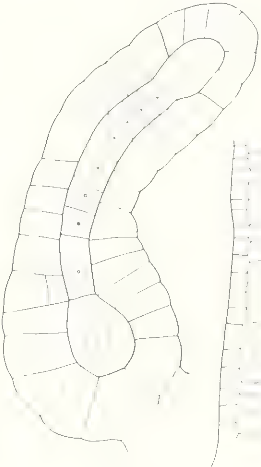
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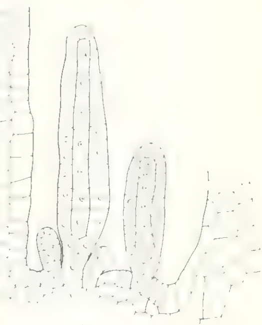
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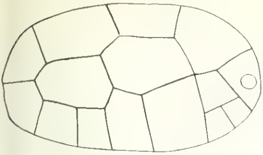
HUTCHINSON on PELLIA



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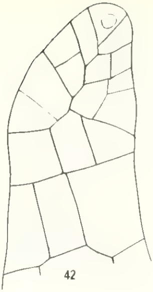

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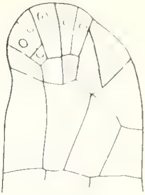

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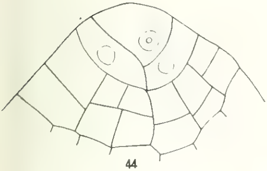

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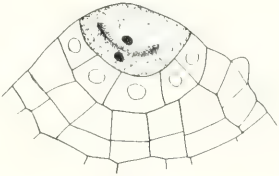

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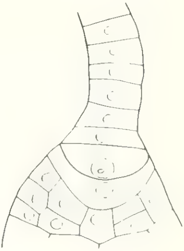

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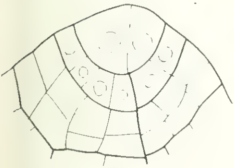

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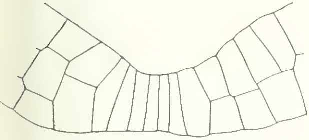

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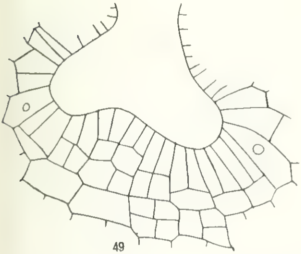

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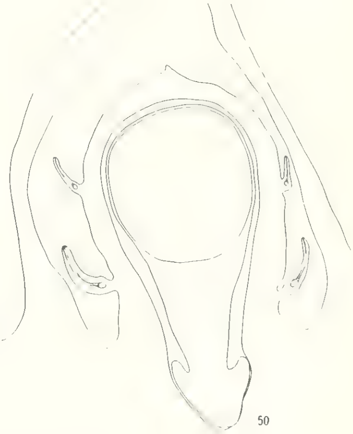

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The University of Chicago

SOME EFFECTS OF ETHYLENE ON THE METABOLISM OF PLANTS

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY

EDWARD MARIS HARVEY

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SOME EFFECTS OF ETHYLENE ON THE METABOLISM OF PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 207

EDWARD MARIS HARVEY

(WITH TWO FIGURES)

Introduction

Etiolated pea seedlings develop abnormally when they are grown in the "impure air" of a laboratory. This response to atmospheric impurities has become well known through the work of NELJUBOW (**25**) and others. Three phases, at least, are usually distinguished in the response of the epicotyl of the seedling: (1) a retardation in the rate of elongation, (2) swelling, and (3) a change from negative geotropism to diageotropism. Furthermore, this characteristic response can be produced by a large number of chemical compounds. However, the three phases mentioned are not induced with equal ease; the third may never appear for a given substance, although that substance readily causes swelling and interferes with the rate of elongation. Likewise, both the second and the third may not appear, although there is a marked retardation of growth. The swelling of developing plant organs in the presence of poisonous substances is a very common response, especially when the concentration of the substance in question is near the lower toxic limit. So frequently does this phenomenon occur that one is perhaps justified in saying that swelling is one of the first superficial indices of a disturbance in the metabolism of a plant.

Of the large number of chemical compounds capable of inducing swelling in the pea seedling, ethylene has been found to be the most effective. According to KNIGHT, ROSE, and CROCKER (**19**), ethylene will cause swelling of the epicotyl of the sweet pea seedling in dilutions of about 0.00004 per cent (by volume), while to produce similar results with chloroform, for example, the concentration

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must be about 1 per cent. This illustrates how sensitive the sweet pea seedling is to traces of ethylene. Moreover, it seems fairly well demonstrated that for plants in general (but by no means all) ethylene is relatively very toxic.

The remarkable capacity of ethylene to induce swelling naturally suggests the question, What is the effect of ethylene on plant metabolism? A certain amount of work has been published on the effects of illuminating gas and "laboratory air," which should furnish required data on the question, since in both those gaseous mixtures ethylene has probably been an important factor. Nevertheless, with regard to the effect of ethylene as such, I have been able to find no literature. This was largely the reason for undertaking the investigation reported here, the work having as its subject the determination of the changes brought about in plant tissue by ethylene.

Historical

Our knowledge of the changes in metabolism causing and accompanying swelling of plant organs has been gained largely from investigation of the effects of anaesthetics, particularly ether and chloroform. For a general historical résumé of the literature of the effects of anaesthetics on plants, the reader is referred to an excellent paper by HEMPEL (**11**). The following consideration of the literature deals only with the effects of anaesthetics on the chemical composition and the respiratory processes.

JOHANNSEN (**16, 17**) found that certain concentrations of ether and chloroform caused an increase of soluble sugars and a decomposition of proteins in bulbs of *Crocus* and seeds of pea and barley. But he also noted that very weak ether gave reversed effects, that is to say, favored starch and protein synthesis. His explanation for the increase in sugars and amino bodies was simply that anaesthetics interfered with the condensation, but not with the hydrolyzing processes. ZALESKI (**36**), working with *Lupinus*, found that protein synthesis was favored by ether and hindered by caffein. BUTKEWITSCH (**3**) and BARTEL (**2**) both reported an increase of tyrosin in *Lupinus*, as an effect of chloroform. PRIANISCHNIKOW (**28**) was able to demonstrate a considerable increase of asparagin in *Lupinus* when the seedlings were grown in an atmosphere containing traces of gaseous impurities. LESCHTSCH (22) studied the effect of turpentine on protein metabolism in bulbs of *Allium*. An acceleration of protein synthesis occurs in wounded bulbs, but the process is further accelerated by small amounts of turpentine and hindered by large amounts. PURIEWITSCH (27) noted that ether interfered with the synthesis of starch. To account for this phenomenon he assumed an increased rate of respiration, whereby the sugars were used up. BUTKEWITSCH (4) reports similar effects for toluol and chloroform. Starch in the bark and wood of *Morus* and *Sophora* was rapidly hydrolyzed. This hydrolysis cannot be explained, he thinks, on the supposition of an increased respiration, since sugars increase concomitantly with the decrease of starch. Also, he points out the analogy between the effects of toluol and chloroform and of low temperatures; both may be explained on the basis of injury to the plastids. REINHARD and SUSCHKOFF (29) determined the effects of several substances upon starch synthesis. Ether seemed to act, not only as a hindrance to starch formation, but also as an accelerator of hydrolysis. Antipyrin, morphine, and caffein hindered, but urea and asparagin favored starch synthesis. Similarly DELEANO (6) observed a rapid destarching of leaves in the presence of chloroform, a result apparently contradictory to that reported by CZAPEK (5). RICHTER (30), working with illuminating gas, laboratory air, xylol, etc., and GRAFE (8), with formaldehyde, have shown that an accumulation of sugar is favored by these substances. ARMSTRONG and ARMSTRONG (1) have demonstrated that toluol, ether, chloroform, etc., cause an increase of glucose and HCN in leaves of *Prunus lauro-cerasus*, due to a rapid splitting of the glucoside present. HEMPEL (11) has made a careful study of the effects of ether on seedlings of *Pisum* and *Lupinus* with particular regard to the CO_2 output, and the changes in the nitrogen compounds and sugars. Her results show that ether effects are dependent upon the concentration. The normal destruction of the proteins in germination was retarded by "weak" doses (up to approximately 0.01 per cent by volume), but the process was accelerated in strong doses. All concentrations interfered with the inversion of sugars. GRAFE and RICHTER (9) have published an article on the effects of acetylene on the chemical composition of several kinds of seeds and shoots. They found that the sugars and amino acids increased in tissues which were naturally high in carbohydrate (*Vicia*, *Laburnum*, and potato shoots), while in fatty tissue (seeds of squash, mustard, and flax) there was a slight decrease. Also, acetylene caused an increase in the amount of glycerine and fatty acid in the seeds, resulting in a decrease in the amount of fat. Like results were obtained for illuminating gas. They conclude that the condensation processes alone are affected. However, it seems quite possible that anaesthetics sometimes also hasten the hydrolyzing processes. This is further indicated by the recent work of McCool (24) in which he claims that the acceleration of enzymatic activity (of diastase and oxidase) takes place during etherization, although the activity of catalase is depressed.

A preliminary examination of the results referred to above shows a number of inconsistencies; but Grafe and Richter have well pointed out that these inconsistencies are probably not real. Most of them become clear when the effect of anaesthetics, with regard to the general chemical reactions of plants, is expressed as follows: that the condensation processes are favored by "weak" and hindered by "strong" concentrations; but that the effect on the hydrolyzing process is uncertain.

The literature dealing with the effects of anaesthetics on respiration processes uncovers about the same general situation as stated above, since weak doses seem to accelerate and strong doses to retard respiration. This statement is borne out by the results of Elfving (7), Johannsen (15), Morkowin (23), Laurén (21), and others. However, Morkowin considers that the respiration of carbohydrates cannot be accelerated by ether; that such is possible only with nitrogenous substances. Also Laurén found that whether or not respiration could be accelerated by ether depended upon the kind of plant used. Respiration was accelerated in proportion to the dose in *Ricinus* and *Lupinus;* slightly accelerated in limited doses, later depressive, in *Pisum*, *Phaseolus*, and *Cucurbita;* and there was no acceleration in *Brassica*, *Hordeum*, and *Zea*. Irving (12) has shown that for chloroform the effect depended upon the dose. Small doses increased the CO_2 releasal; medium doses caused an initial outburst, afterward a falling off; and strong doses caused depression from the beginning. THODAY (33) has made some careful determinations, both of CO_2 releasal and of O_2 absorption. Weak concentration of chloroform accelerated both processes to an equal degree, hence the respiration ratio remained the same. When the doses were considerably stronger, respiration was retarded, but the correlation between the two processes was broken up. In leaves without tannin (*Tropaeolum*) O_2 absorption was depressed more than the CO_2 output. But in leaves containing tannin (*Pinus* and *Helianthus*) the situation was reversed; there was an initial rapid absorption of O_2 which soon fell to a level somewhat above the CO_2 production.

Material

The sweet pea seedling was chosen as experimental material for the present study, largely because it is so sensitive to toxic substances, and on account of the general interest surrounding its characteristic responses.

Etiolated seedlings were used throughout the experiments. The seeds were purchased under the trade name Gladys Unwin (Vaughn's Seed Store). The cultural methods employed have been described by KNIGHT and CROCKER (20), although some minor changes were necessary in order to care for large cultures. The methods are briefly outlined below.

The seeds were scratched with a file (to secure quick and uniform germination), soaked for 12 hours in distilled water, and germinated on wet filter paper. When the hypocotyls had become 3–7 cm. long the seeds were sowed upon wet absorbent cotton in large pans ($2\times30\times48$ cm.) and covered with a layer of wet filter paper. They were allowed to develop in absolute darkness at a temperature of 21–24° C., until the epicotyls had reached an average length of about 2 cm. The filter paper was then taken off the seedlings and the culture equally divided into two portions, one for treatment with ethylene, the other for control. The entire culture usually consisted of 12 pans, each containing about 250 seedlings. The portion for ethylene treatment was transferred to a galvanized iron box of 225 liters' capacity; the lid sealed gastight; and enough ethylene[1] admitted to make the concentration about 0.0001 per cent by volume. Both control and treated portions were then allowed to continue development for 72 hours under the same conditions (that is, of moisture, temperature, and darkness), except for the ethylene in one. At the end of this period the epicotyls were collected for experimentation. The total culture period was 9 days.

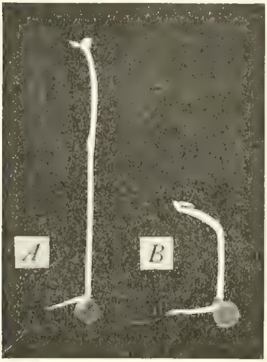

FIG. 1.—Etiolated seedlings of the sweet pea, showing the stage of development at which the epicotyls were taken for experimentation: *A*, normal; *B*, ethylene treated, showing the "triple response"; $\times\frac{1}{2}$.

The epicotyls of the control seedlings, at the time of collection, were 8–11 cm. in length and vertical and straight. But the ethylene treated seedlings showed the well known "horizontal nutation" (NELJUBOW **25**) or "triple response" (KNIGHT and CROCKER); that is, the epicotyls were only 3–5 cm. long, swollen, and had assumed horizontal or nearly horizontal positions. The difference in appearance between the ethylene treated and the untreated seedlings is shown in fig. 1. Certain histological differences are shown by the drawings of fig. 2.

Methods and experimentation

The present attack of the problem on the effects of ethylene has been made through a study of the following questions: (1) chemical composition, (2) acidity, (3) osmotic pressure and permeability, and (4) respiration. A decided emphasis has been laid upon the chemical phase of the problem.

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[1] The ethylene used in these experiments was prepared by dropping ethyl alcohol into syrupy phosphoric acid at a temperature of about 215° C. The final dilutions of ethylene were made from a stock ethylene-air mixture containing 2.5 per cent ethylene.

A. CHEMICAL ANALYSIS

1. *General procedure*

At the close of the culture period, the epicotyls of the seedlings were collected, their wet weight determined, and preserved in 85 per cent alcohol[2] (redistilled). Both the ethylene treated and the untreated samples usually weighed 100-150 gm. Soon after

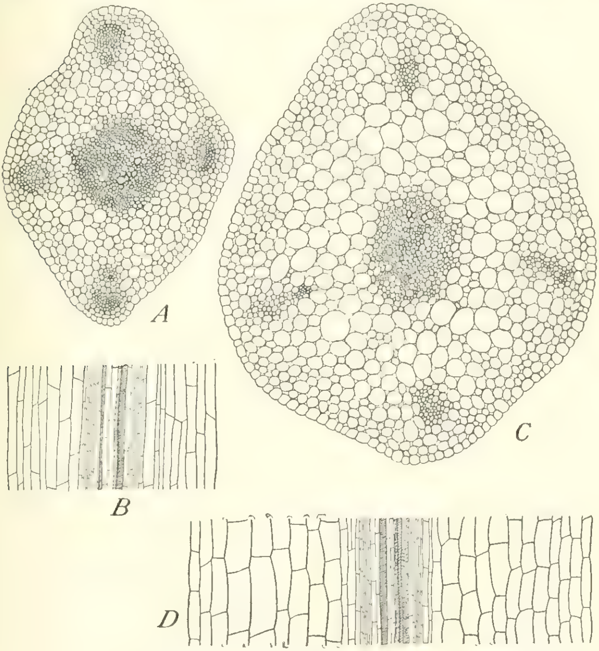

FIG. 2.—Sections of the sweet pea epicotyl at the stage corresponding to fig. 1: *A* and *B*, normal; *C* and *D*, ethylene treated; ×50.

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[2] Enough 95 per cent alcohol was added to make the final concentration 85 per cent, allowing for water in the tissue, which was about 92 per cent.

preserving, the tissue was heated in a water bath for one hour at 70° C. and set aside for at least one week before proceeding with the analysis. Later the epicotyls were cut with scissors into 2 5 mm. lengths, transferred to ashless filter paper extraction cups, and the preserving liquid filtered through the cups. Two extractions followed, one with hot 95 per cent alcohol for 4 hours and the other with hot ether for 2 hours. Then the tissue was powdered in a mortar and finally extracted for 12 hours more with hot 95 per cent alcohol. This procedure separated the sample into alcohol-ether soluble and insoluble portions. The dry weight of each was determined by methods which are described below. That of the insoluble fraction was found simply by drying to constant weight in an oven at 104° C. But the soluble fraction was concentrated upon a water bath to about 450 cc., transferred to a 500 cc. volumetric flask, and made up to the mark. Then an aliquot part (usually 100 cc.) was taken for dry weight determination, and later for ashing. The final drying was carried out in a vacuum desiccator over $CaCl_2$.

Analysis of the alcohol-ether soluble fraction was carried out on the remaining 400 cc. This was evaporated to small volume to free it from alcohol, taken up with water, and transferred to a 500 cc. volumetric flask. Fats and lipoids were precipitated from solution by the addition of 3 5 cc. of chloroform and 10–15 cc. of 5 N HC. After shaking, the solution was made up to volume and set aside in an ice box for 24 hours to settle. The clear supernatant liquid was then decanted and filtered. No determinations were made upon this fat and lipoid residue. The water solution was divided into portions for the following determinations: (*a*) carbohydrates, (*b*) total nitrogen, and (*c*) ammonia and alpha-NH_2 nitrogen.

Analyses on the alcohol-ether insoluble fraction included the following: (*a*) reducing sugars after acid hydrolysis, (*b*) total nitrogen of the fraction, (*c*) total nitrogen of the portion rendered soluble by acid hydrolysis, (*d*) "crude fiber" (dry weight only), which was that portion remaining insoluble after acid hydrolysis, and (*e*) weight of ash. The "crude fiber" would largely be cellulose plus protein which was yet undissolved by acid hydrolysis, hence the results from $(d)-[(b)-(c)]$ should give an approximate figure for the cellulose present.

2. *Carbohydrates*

Reducing sugars, before and after hydrolysis, were determined for the alcohol-ether soluble fraction. The methods employed were those of MUNSON and WALKER (**26**, pp. 241–251). The reduced copper was determined by the volumetric permanganate method described in the above-named bulletin (p. 52). Similarly, the reducing sugars were determined for the insoluble fraction after 2.5 hours of hydrolysis with 3.5 per cent HCl.

3. *Nitrogenous substances*

Total nitrogen was determined by the KJELDAHL method and the amino acids and amids by both the formol titration (as described by JESSEN-HANSEN, **14**, and VAN SLYKE's methods, **34**). While employing the latter method, estimations were made of ammonia and alpha-NH_2 groups both before and after a 24-hour hydrolysis with 20 per cent HCl at 98–99° C. (see **35**).

4. *Fats*

Only the dry weight of the ether extract and the free fatty acid value were estimated. Separate samples were used for fat determinations. The tissue was collected and preserved as previously described, but instead of the alcohol extraction being used as before, the tissue was spread out to dry in a current of air, absolute alcohol being added from time to time to hasten the drying process. The dried tissue was then extracted for 12 hours with absolute ether. Also, the preserving liquid was evaporated, the residue dried *in vacuo*, and taken up with absolute ether. The ether extracts were combined, dried *in vacuo*, and the weight determined. Free fatty acids of this residue were estimated by titration in the usual manner.

5. *Results*

The following statements are made in explanation of the tables of results. Whenever percentages are given, the figures are always in terms of the *total dry* weight of the samples. Also, the numbers of the samples are given in order to facilitate proper comparison between ethylene treated and untreated tissue; for, as has been stated, a given culture was not divided into the two portions until 72 hours before the end of the cultural period. Considering the unavoidable variations which must enter into different cultures, the treated and untreated pair of samples from one culture should therefore be more comparable than any other combination; for example, the treated sample of one culture and the untreated of another. Samples I and II, afterward IV and V, VI and VII, etc., are directly comparable.

TABLE I

TOTAL ALCOHOL-ETHER SOLUBLE SUBSTANCES IN THE ETHYLENE TREATED AND UNTREATED TISSUE

UNTREATED				TREATED			
No of sample	Percentage of alcohol-ether soluble	Percentage of alcohol-ether insoluble	Percentage of total solids	No of sample	Percentage of alcohol-ether soluble	Percentage of alcohol-ether insoluble	Percentage of total solids
I.	59.79	40.21	8.73	II.	67.63	32.37	8.54
V.	59.28	40.72	8.00	IV.	68.80	31.20	7.82
VII.	59.04	40.96	7.82	VI.	67.43	32.57	8.32
XIII. . .	59.40	40.60	8.50	XII.	64.98	35.02	8.60
XV.	60.00	40.00	7.80	XIV.	67.60	32.40	8.30
XIX.	59.60	40.40	8.34	XVIII (*a*)	68.64	31.36	8.90
XXI. . . .	59.70	40.30	8.53	XVIII (*b*)	68.70	31.34	8.94
XXIII . .	59.66	40.37	8.48				
Mean . .	59.55	40.45	8.27	Mean.	67.70	32.30	8.48

Table I gives the results of the separation of the samples into alcohol-ether soluble and insoluble portions. Two facts are noteworthy: first, that the soluble substances are more abundant in the ethylene treated than in the untreated tissue, a difference amounting to about 8 per cent; and, secondly, the water content of the two tissues is practically the same, being about 91.5 per cent. To what substances this difference is due should become clearer from the tables to follow.

Table II shows the amount of reducing sugars in the alcohol-ether soluble fraction before and after hydrolysis, and also the reducing power of the insoluble fraction after hydrolysis. These latter data cannot be expressed, even approximately, in percentages, on account of the probability of a great variety of hydrolyzable polysaccharides present.

The amount of reducing sugars in the soluble fraction is considerably greater in the treated tissue, although the results of sample II are inconsistent. Again, after hydrolysis, the treated tissue still shows more reducing sugar, but the difference is less pronounced, which means that the higher soluble carbohydrates, such as the disaccharides, are really less in this tissue than in the untreated. One seems justified in saying that the ethylene treated tissue has about 11 per cent more of the lower, and about 3 per cent less of the higher soluble sugars than the untreated. The reducing power of the alcohol-ether insoluble fraction after hydrolysis is clearly less in the treated tissue. The polysaccharides, which are likely to be present and which are capable of yielding reducing sugars by this acid hydrolysis, are starch, ligno-celluloses, galactans, pectins, etc. Microchemical tests show that very little starch is present in either tissue. The reducing sugars, therefore, are largely from other polysaccharides. An examination of the drawings of fig. 2 will aid in interpreting the differences found. Around the four leaf traces, mechanical tissue is considerably more abundantly

TABLE II

CARBOHYDRATES

	Alcohol-ether soluble fraction				Alcohol-ether insoluble fraction
	No. of sample	Percentage of reducing sugars before hydrolysis	Percentage of reducing sugars after hydrolysis	Increase by hydrolysis	Mg. cu. reduced for each gm. of material after 2.5 hours' hydrolysis
Untreated tissue	I	....	....	....	153.0
	V	....	....	....	102.2
	VII	10.10	15.02	5.83	143.4
	XIII (*a*)	....	....	....	131.4
	XIII (*b*)	....	....	....	145.2*
	XV	12.30	15.70	3.40	140.1
Ethylene treated tissue	II	13.84	15.15	1.31	120.7
	IV	23.18	25.56	2.38	124.6
	VI	23.70	25.30	1.60	109.7
	XIV	20.39	21.51	1.12	118.8

* Hydrolysis 5 hours.

developed in the untreated epicotyl. This anatomical difference agrees with the findings of KAUFMANN (18) for lupine seedlings treated with ether. From a chemical viewpoint the difference seems sufficient to account for the greater power of reduction of the untreated tissue after hydrolysis, inasmuch as mechanical tissue contains a large proportion of rather easily hydrolyzable carbohydrates. That these polysaccharides are not completely hydrolyzed by the 2.5 hours' hydrolysis is shown by comparing samples XIII (*a*) and XIII (*b*), in which the hydrolyzing time of the latter was doubled.

TABLE III

	No. of sample	Percentage of "crude fiber"	Approximate percentage of cellulose
Untreated tissue	V	15.09	9.2
	VII	16.18	11.4
	XIII (*a*)	15.00	....
	XIII (*b*)	13.94*	....
	XV	15.05	....
Ethylene treated tissue	II	9.22	....
	IV	11.12	6.22
	VI	11.28	8.3
	XIV	11.66	....

* Hydrolysis 5 hours.

Table III gives the results of "crude fiber" determinations. "Crude fiber" obviously is a mixture of a large number of substances, such as unhydrolyzed protein, cellulose and other polysaccharides, etc. However, this fiber must be largely cellulose and protein. Less crude fiber was found in the treated tissue. The approximate percentage of cellulose was estimated by subtracting from "crude fiber" the total protein present before hydrolysis, minus the protein rendered soluble by the acid hydrolysis. By this method relative differences, at least, should be shown, and the results indicate that the treated tissue had about 3 per cent less cellulose than the control.

In table IV are shown the total nitrogen and the ammonia and amino nitrogen, before and after hydrolysis, in the alcohol-ether soluble fraction, and the total nitrogen in the alcohol-ether insoluble fraction.

The total nitrogen figures for the former fraction do not show a difference between the treated and untreated tissues. Both the formol titration and the Van Slyke methods show a higher amino nitrogen content in the treated tissue before hydrolysis, but after hydrolysis the difference does not appear. (Determinations after hydrolysis were made only by the Van Slyke method.) Since the increase of alpha-NH_2 nitrogen, by hydrolysis, is somewhat more in the untreated tissue, it suggests a less amount of polypeptides in the treated tissue, a situation for the soluble nitrogenous substances corresponding to that of the soluble carbohydrates of this fraction. The ammonia-nitrogen results are very variable and no conclusions can be drawn from them.

TABLE IV

Nitrogen

	Alcohol-ether soluble fraction						Alcohol-ether insoluble fraction	
	No. of sample	Total nitrogen	NH_3 before hydrolysis	Amino-nitrogen before hydrolysis	NH_3 after hydrolysis	Amino-nitrogen after hydrolysis	Total nitrogen	Protein
Untreated tissue.....	I	5.18	....	....	.....	.....	2.19	13.68
	V	5.41	......	......	.....	.....	1.91	11.93
	VII	5.07	......	......	.....	.....	1.50	9.38
	XIII	......	......	1.62*	.....	.....	.....	......
	XV	......	.....	1.71*	.....	.....	.....	......
	XIX	......	0.32	1.96	.....	.....	.....	.....
	XXI	......	.....	1.93	.....	2.89	......	.....
	XXIII	......	0.76	2.02	1.30	2.78	.....	......
Ethylene treated tissue.....	II	5.20	......	....	.....	....	1.82	11.37
	IV	5.62	.....	...	.....	....	1.57	9.81
	VI	5.20	....	....	.....	.....	0.95	5.93
	XII	......	.....	2.25*	.....	.....	....	.....
	XIV	.....	....	2.34*	....	.....	......	......
	XVIII (*a*)	.....	0.83	2.54	0.93	3.22	.....	......
	XVIII (*b*)	......	0.40	2.41	1.25	2.98	......	.....

*Obtained by the formal-titration method.

The total nitrogen of the alcohol-ether insoluble fraction is less in the treated tissue. By employing the factor 6.25 to the nitrogen figures, it is found that the proteins are about 3 per cent less in the treated tissue. Although the results at first seem somewhat inconsistent, they no longer appear so when treated and untreated samples of the same culture are compared.

Table V gives the percentage of ash of the soluble and insoluble fractions. No marked difference appears between the two tissues.

TABLE V

ASH

	No. of sample	Percentage of ash of the alcohol-ether extract	Percentage of ash of the alcohol-ether insoluble residue
Untreated tissue	I	....	1.58
	V	....	1.73
	VII	3.08	1.35
Ethylene treated tissue............	II	2.76	1.54
	IV	3.15	1.41
	VI	3.22	1.47

Table VI shows the amount of ether soluble substance and the free fatty acid value. The figures in parentheses were not determined, but calculated on the assumption that the percentage of ether-extractives was the same in these samples as in others. One thing is rather clear from the table, namely that less fat is present in the treated tissue, a fact which agrees with the effects of acetylene in oily seeds as studied by GRAFE and RICHTER (9). The free acid value is of particular interest on account of the claim of IWANOW (13) that the free acid value is predetermined by the degree of saturation of the fatty acids involved in the fat in question. However, the free acid value of the fats in the two tissues was not found

TABLE VI

FATS

	No. of sample	Wet wt. of tissue gm.	Dry wt. of tissue gm.	Dry wt. of ether-extract gm.	Percentage of ether-extractives	cc. of N/10 NaOH to neutralize free fatty acid	cc of N/10 NaOH to neutralize free fatty acid in 1 gm. of ether extract
Untreated tissue......	XXVII	119.57	10.043	(0.220)	(2.2)	3.00	(13.63)
	XXIX	171.64	14.417	0.313	2.17	3.86	12.33
Ethylene treated tissue ...	XXVI	139.45	11.713	(0.140)	(1.2)	1.92	(13.71)
	XXVIII	264.55	22.222	0.271	1.22	3.50	13.13

to differ. On the foregoing assumption one may therefore say the nature of the fats in the treated and untreated tissues is the same as regards degree of saturation.

B. ACIDITY

For acidity determinations the epicotyls were collected as described for the chemical analysis. The wet weight of the sample was determined, but instead of being preserved in alcohol, the tissue was directly triturated with water in a mortar. More water was added to bring the mixture up to a definite volume, and finally the free acids present were titrated with N/10 NaOH, using phenolphthalein as indicator. The entire procedure, from the cutting of the seedlings to the end of the titration, required about one hour. The foregoing method is rather unsatisfactory; in addition to the fact that in this way one estimates only the surplus H-ions, other objections may be offered. However, any marked relative difference can be caught by this method.

TABLE VII

ACIDITY

	Wet. wt. of tissue in gm	cc. of N/10 NaOH to neutralize 1 gm. wet wt. of tissue
Untreated..........	87.25	0.7241 0.7482
	25.65	0.8457 0.8730
Ethylene treated....	79.65	0.7706 0.7313
	29.55	0.8288 0.8118

The results obtained are found in table VII, expressed in terms of N/10 NaOH required to neutralize 1 gm. wet weight of the tissue. No consistent difference is evident between the treated and control tissues.

C. OSMOTIC PRESSURE AND PERMEABILITY

Osmotic pressure was estimated by two methods, freezing point and plasmolysis. For the former method, the juice was expressed by means of a hand press giving about 300 kgm. per sq. cm., the tissue having been coarsely cut up with scissors and wrapped in a single layer of art canvas. The freezing point was determined with the BECKMAN apparatus, following the directions given by HAMBURGER (**10**). Both osmotic pressure and permeability were investigated by the plasmolytic method. Plasmolysis was observed in the cortical cells just underlying the epidermis at the base of the second leaf scale (that is, second from cotyledons). Plasmolytic agents employed were sucrose, glucose, KNO_3, and glycerine. A solution was considered isotonic with the cell sap if it just caused plasmolysis after 30 minutes. The temperature was 20–24° C.

TABLE VIII

OSMOTIC PRESSURE BY FREEZING POINT

	No. of sample	Δ	Pressure in atmospheres	Mean pressure
Untreated	V	0.610	7.33	
	VII	0.632	7.60	
	XI	0.703	8.46	
				7.79
Ethylene treated	IV	0.755	9.08	
	VI	0.818	9.84	
	VIII	0.827	9.94	
	X	0.782	9.41	
	XIV	0.821	9.87	
				9.63

In table VIII are the results by the freezing point method. It is evident that the juice of the treated tissue has a higher osmotic pressure than that of the control, a difference of about two atmospheres.

Similarly, table IX gives the results by the plasmolytic method. The figures show that the same relative difference of about two atmospheres exists between the treated and untreated tissues, although the pressures themselves are somewhat higher. RICHTER (**31**) and others have assumed a rise of osmotic pressure in tissues under the influence of anaesthetics. This assumption is based upon the fact that sugars and other osmotically active substances were known to increase. However, no previous measurements of osmotic pressure under such conditions have apparently been made.

Results with KNO_3 and glycerine indicate probably two things: first, that neither the treated nor the untreated tissues are very permeable; and, secondly, that the treated tissue is slightly more permeable than the control.

TABLE IX

OSMOTIC PRESSURE AND PERMEABILITY

	Plasmolyzing agent	Concentration gm. mol.	20–24° C. Osmotic pressure in atmospheres	Difference in gm.-mol between treated and untreated
Untreated........	Sucrose	0.37	9.38–9.52	....
	Glucose	0.37	....	....
	KNO	0.21	....	....
	Glycerine	0.43	....	....
Ethylene treated..	Sucrose	0.46	11.70–11.84	0.09
	Glucose	0.47	....	0.10
	KNO	0.29	....	0.08
	Glycerine	0.56	....	0.13

D. RESPIRATORY CHANGES

For the study of respiration, the cultural methods differed in some respects from those already described. When the epicotyls had become 2–3 cm. long (that is, ready for the usual 72 hours' exposure period), the entire seedlings or the epicotyls only were taken from the pans and placed in test tubes of 20 cc. capacity, graduated for 15 cc. The condition in the test tubes were as follows: They were filled with mercury and inverted over a dish of the same. The mercury in the tubes was displaced to the 15 cc. mark, either with pure air or an ethylene-air mixture containing 0.0002 per cent ethylene. Three entire seedlings or four epicotyls were introduced from below into the various tubes. The experimental periods were 3, 6, 12, 24, 48, and 72 hours. At the close of a period the seedling or epicotyls were withdrawn by means of a hooked wire and the gas present preserved for analysis. The BONNIER and MANGIN apparatus was employed for the gas analysis, following practically the procedure suggested by THODAY (32).

In table X are the results of the analyses. Each set of figures represents the average of a number of analyses; for example, the 12-hour cultures are from 16 analyses upon gas of 3 different cultures. The results plainly show a general depression of respiration by ethylene, both in the CO_2 production and the O_2 absorption. The respiratory ratio gradually increases with the time in both tissues, an increase which probably is due to the lowering of the oxygen pressure. In the 3-hour culture with ethylene the ratio is very large. The result, as it stands, comes from an excessive production of carbon dioxide. This ratio of 0.81 seems extremely high in consideration of the 0.66 ratio of the control, and particularly of the 0.61 ratio of the 6-hour ethylene culture. However, IRVING (**12**) in her study of the effects of chloroform on barley leaves found that "medium" doses cause a large initial outburst of CO_2 quickly followed by a depression.

TABLE X

RESPIRATORY CHANGES

No. of hours		UNTREATED TISSUE			ETHYLENE TREATED			
		cc. CO_2	cc. O_2	$\frac{CO_2}{O_2}$	Concentration of gas used, percentage (by vol.)	cc. CO_2	cc. O_2	$\frac{CO_2}{O_2}$
3	Epicotyls only	0.2185	0.3664	0.66	0.0002	0.2449	0.3030	0.81
6	" "	0.2255	0.3419	0.66	"	0.1703	0.2898	0.61
6	Entire seedling	0.4138	0.6069	0.69	"	0.3780	0.5744	0.66
12	" "	0.3758	0.5345	0.70	"	0.3197	0.4668	0.69
24	" "	0.3587	0.4190	0.84	"	0.3127	0.3873	0.81
24	Epicotyls only	0.3145	0.4025	0.78	0.01	0.3380	0.4086	0.84
48	Entire seedling	0.2501	0.2897	0.86	0.0002	0.2404	0.2869	0.85
72	" "	0.3210	0.2300	1.39	"	0.2610	0.2212	1.04

Conclusion

The results of the present study seem to indicate that the general effect of ethylene on plant metabolism is exactly comparable to the effects of the common anaesthetics, chloroform, ether, etc., as reported by other workers. Also, that the 0.0001 per cent ethylene concentration used is equivalent in its physiological effects to the so-called "strong" concentrations of those anaesthetics, concentrations which are in reality, for example in the case of chloroform or ether, thousands of times stronger. Probably most of the ether concentrations employed by HEMPEL (11) were many times weaker, physiologically, than the ethylene concentrations of the above-described experiments. Such an assumption would account for the difference between some of her results and those reported in this paper. It seems probable that ethylene, also, would favor condensation processes if used in "weak" or "medium" concentrations.

In the presence of ethylene the simple soluble substances increase at the expense of the higher soluble and insoluble forms; direct reducing sugars against soluble non-reducing sugars and insoluble polysaccharides; amino acids and amids against proteins; and probably fatty acids and glycerine against fats, seeing that the latter were found to diminish. Accordingly, ethylene appears to affect the balance of the general chemical reactions of the plant in favor of the simpler substances. The experimental work offers no evidence as to whether or not this result is accomplished through an acceleration of the hydrolytic as well as through a retardation of the condensation processes, since all the substances present in the tissue examined (epicotyls) had, within a relatively few hours, arrived, in simple translocation forms, from the cotyledons.

The accumulation of soluble substances in the tissue changes the osmotic relations of the cells and may have much to do with the observed swelling of plant organs in the presence of ethylene, for example in the characteristic "horizontal nutation" or "triple response" of the pea epicotyl. Also, the observed retardation of the rate of elongation may partly be accounted for by the fact that the gas interferes with the synthesis of complex substances, that is to say, perhaps with tissue formation.

Summary

1. Ethylene was found to be very effective in producing changes in the general processes of plant metabolism.

2. Chemical analyses showed that ethylene caused the simple soluble substances to increase at the expense of the higher soluble and insoluble forms.

a) The hot alcohol-ether soluble substances (sugars, amino acids, amids, polypeptides, lipoids, etc.) increased by 8 9 per cent, while the insoluble substances (proteins, starch, cellulose, lignocelluloses, etc.) were correspondingly diminished. The water content of the ethylene treated and control tissues was the same.

b) The lower soluble sugars (by direct reduction) were about 11 per cent more and the higher soluble sugars (by reduction after hydrolysis) about 3 per cent less. The reducing power of the alcohol-ether insoluble residue, after hydrolysis, was decidedly less for the ethylene treated tissue; also, the cellulose content was diminished by about 3 per cent.

c) Amino acid plus amids were more, and the polypeptides apparently less in the ethylene treated tissue. The protein content also was about 3 per cent less.

d) Fats were much less abundant in the treated tissue. The free fatty acid value was unchanged.

3. The acidity of the ethylene treated tissue was not found to be changed.

4. Ethylene caused an increase of osmotic pressure, as measured both by the freezing point and plasmolytic methods.

5. The permeability was not sharply affected by ethylene, although it was somewhat increased.

6. Ethylene affected respiration, retarding both the CO_2 production and the O_2 absorption, but the respiratory ratio remained practically the same. An exception to the preceding statement was found in the case of the shortest exposure period (3 hours), in which there occurred, apparently, an excessive production of CO_2, thereby increasing the ratio.

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VASCULAR ANATOMY OF THE MEGASPOROPHYLLS OF CONIFERS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 208

(WITH 196 FIGURES)

HANNAH C. AASE

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(WITH 196 FIGURES)

Introductory and historical

The megasporophyll in Coniferales has been the subject of much investigation and discussion, as on the interpretation of this structure depends to a great extent the views held in regard to the relation of living conifers to fossil forms, and the interrelation of living genera. If the scale in *Pinus*, the ligule in *Araucaria*, and the epimatium in *Podocarpus* represent a dorsal outgrowth of the bract, there is added a strong argument in support of the contention that the conifers have sprung from lycopod stock. If the megasporophyll represents a metamorphosed fertile shoot and its subtending bract, there exists a suggestive likeness to the Cordaitales, in which the presence of bracts on the shoot makes its identification as a shoot less difficult. Again, if the scale in the Abietineae represents an axillary shoot and the ligule in *Araucaria* represents a dorsal outgrowth, two other possibilities may be suggested: either the Coniferales have a double origin, or the Cordaitales included not only forms with compound strobili in which the scale is a metamorphosed structure, but also forms with simple strobili in which the scale is a ligular outgrowth of the bract. All these four views are supported by various investigators. Correlative with the views taken as to the origin of the group as a whole are the views as to the interrelation of genera; forms which may be considered as progenitors according to one theory may be the descendants according to another, or there may exist no relation.

The investigators before 1868 were concerned chiefly with gross observations of development and abnormalities. Rather complete summaries of these investigations are given by RADAIS (**2**) and WORSDELL (**11**).

VAN TIEGHEM (**9**) in 1869 was the first to attack the problem from the standpoint of vascular anatomy. He studied forms from all the six large groups. He concluded that the megasporophyll in all Coniferales is a compound structure. The seminiferous scale represents the first and only leaf of an axillary shoot, as the vascular supply to the scale is arranged in an arc. The ovules are borne on the dorsal side of the leaf except in *Araucaria*, where the ovule is reflexed toward the ventral side and hence appears to be located between the bract and scale. In the Podocarpineae and Taxineae the leaf is reduced to such an extent that it is represented practically only by the ovule. In *Podocarpus* the leaf is folded on its dorsal surface to form an anatropous ovule, while in *Pherosphaera*, species of *Dacrydium*, *Phyllocladus*, and the Taxineae, the leaf remains erect and the ovule is orthotropous. The inversion of the ovule in certain forms is probably related to the greater elongation of the sporophyll beneath the ovular insertion in those forms.

STRASBURGER (**6, 7**) in 1872 and 1879 gave comprehensive descriptions of forms from all the groups. He held that in all cases the ovule-bearing organ is an axillary structure. In *Taxus* and *Torreya* the ovule is borne at the end of a secondary leafy shoot; in *Cephalotaxus* the secondary shoots are reduced to ovules. In the podocarps the secondary shoot is leafless and often reduced to an ovule as in *Phyllocladus*, or provided with a stalk as in *Dacrydium* and *Podocarpus*. In the Araucarineae it appears as if a stalk bearing an inverted ovule were fused to the dorsal side of the bract. In *Cunninghamia*, which he classified with the Araucarineae, there is a fusion of an inflorescence to the bract. In the Abietineae the scale is a flattened axillary structure which is folded inward and hence bears the ovules inverted. The two ovules suggest that the axillary shoot is an inflorescence, a primary and two secondary shoots similar to the two-flowered inflorescence in *Cephalotaxus*. In the Cupressineae and Taxodineae the scale and bract are fully welded together. Where many ovules occur, as in *Cupressus*, he left it undecided whether the ovules represent a reduced branch system, or the large number of ovules is a new feature.

RADAIS (**2**) in 1894 made a rather intensive study of a number of cones of the Abietineae and Taxodineae. He notes that the bundles to bract and scale are distinct in origin in the Abietineae, *Sciadopitys*, and some of the Taxodineae, as *Cryptomeria*, *Taxodium*, and *Sequoia*, and how this distinctness is on its way to obliteration in species of *Arthrotaxis* and more so in *Cunninghamia*, and is lost in *Araucaria Rulei*.

WORSDELL (**10, 11**) in 1899 made a comparative study of types from the different tribes. He believes that in the megasporophyll in all conifers there is an axillary structure concerned. Speaking of *Araucaria* he says:

> Holding to the theory of the axillary bud as the explanation of the structure of the appendage of the cone in *Araucaria*, I believe, with CELAKOVSKY, that the ligule represents the seminiferous scale which is itself the vegetatively developed outer integument of a sporangium situated in the anterior position on an axillary bud. This outer integument has become almost completely fused with the subtending bract in *Araucaria*, completely so in *Agathis*.

Concerning the Taxeae and Podocarpeae he says:

> The Taxeae differ from the other groups in the fact that the sporangia occur in a position terminal instead of lateral to the axis on which they are borne. The anatomy points clearly to the fact that no axial foliar appendage of any kind exists upon which the sporangia are inserted, the cylinder of the axis being directly continuous into the base of the sporangium. This latter difference, however, amounts to very little if we regard, with CELAKOVSKY, the seminiferous scale of the other groups as being the morphological equivalent of the outer integument of the Taxeae, which has become, with the exception of Podocarpeae, vegetatively developed. In the Podocarpeae the relationship is precisely the same as in the Taxeae, with the exception of the axillary instead of terminal position of the sporangium. In this order the bundle system belonging to the sporangium (which is in all the other groups the sole representative of the sporophyll according to the view I here adopt) becomes obvious, owing to the fact that the latter gets by the basal intercalary growth on to the upper part of the bract. In the four other groups the bundle system pertaining to the vegetative development of their outer integuments, which, in the form of the widely expanded seminiferous scale, possesses a pronounced vascular tissue.

SEWARD and FORD (**3**) in 1906, in a somewhat extensive article on living and fossil Araucarineae, offer no interpretation of the megasporophyll in the Abietineae, but in the Araucarineae they consider it as a simple structure which may or may not be homologous with the double structure in other conifers. They regard the Araucarineae as one of the oldest if not the oldest of the conifers. They favor the lycopod origin of the Araucarineae and set them apart under the name of Araucariales.

THOMSON (8) in 1909, in a paper on *Saxegothaea* and *Microcachrys*, admits that the brachyblast theory is inevitable in the Abietineae, Taxodineae, and Cupressineae. Accepting BRAUN'S conception that the scale in the Abietineae represents the first and only two leaves of an abortive shoot, which have fused by their adaxial margins, he says:

> The first inversion is explained and the ovules in the group are borne on the morphological under side. The second inversion is analogous to the single one in *Saxegothaea* and of the nature of a sporangial supply. There are then two great groups of conifers from the standpoint of this study, the simple and the complex scaled series. Both forms have the ovules on the physiologically upper surface, a position rendered almost imperative by the necessities of the seed habit. This position however has been attained in two very different ways.

STILES (5) in 1912 investigated several species of *Podocarpus*. He concludes that the original position of the ovule was erect and axillary as in *Pherosphaera*, but that owing to growth of the scale at the base of the sporophyll it has been carried away from the axis. As a result it has become inverted, and correlated with the inversion is probably the development of an incomplete epimatium.

> Whether this epimatium is an outgrowth of ovular or sporophyll tissue it is at present impossible to say. The evidence of development in *Saxegothaea* and *Microcachrys* suggests the former, while a somewhat older state in *Dacrydium cupressinum* suggests the latter.

In the latter form the ovule is borne on the epimatium, while in *Podocarpus* the epimatium has elongated into a stalk. The development of a strong and independent vascular supply in the epimatium he thinks is the result of a required need of a larger ovule. The epimatium in the podocarps and the scale in the Abietineae are homologous, but both are new structures. Both these complicated structures have been derived from a simple sporophyll.

The Abietineae and Podocarpineae have come from a common primitive stock. The Abietineae are more advanced in the development of the scale, but more primitive in holding on to a perfect cone. "The evidence at present is much in favor of the lycopodean ancestry of the conifers." He has little faith in the brachyblast theory, as it depends for its support mostly on abnormalities and the vascular anatomy of the cone scales. "But abnormalities, especially when they are supposed to be more or less of the nature of reversions, afford by themselves unsatisfactory evidence of phylogeny." Vascular anatomy disproves the double nature of the megasporophyll of the araucarians and podocarps, except in some species in the latter, and there the compound structure is of recent origin.

SINNOTT (4) in 1913 gave a very clear account of the strobilar anatomy in a number of podocarps. He is of the opinion that the podocarps and araucarians, along somewhat parallel lines of development, have been evolved from ancient abietinean stock. The scale in the Abietineae, the ligule in the araucarians, and the epimatium in the podocarps are all homologous and vestiges of an axillary shoot, and a simple sporophyll has arisen either by the fusion of both of its parts or by the abortion of one. Of the podocarps he considers those most primitive in which the epimatium is well developed and has a strong vascular supply, as *Podocarpus;* and those most advanced in which there is a reduced epimatium, as *Dacrydium.* In *Podocarpus dacrydiodes* there is a definite step in the direction of *Saxegothaea, Microcachrys,* and *Pherosphaera.* The resemblance in reproductive structures between certain members of the Podocarpineae and *Cephalotaxus,* the most primitive genus of the Taxineae, suggests that the latter family has arisen from some ancient member of the Podocarpineae.

EAMES (1) in 1913, in a paper on *Agathis,* considered also the megasporophyll situation in other conifer groups and concludes that the megasporophyll is compound in origin in all Coniferales. "Even within themselves the Araucarineae show a complete series from a form with strobilar units of a distinctly double nature to one most simple through reduction." EAMES has traced a similar reduction in the Taxodineae.

Investigation

ABIETINEAE

The ovulate strobilus in all the Abietineae is composed of a comparatively large number of sporophylls. The sporophyll here is obviously composed of two organs. In some forms, as *Keteleeria* and *Pseudotsuga*, each of the three-pronged bracts is reflexed over the scales below, giving the strobilus a bristly appearance; while in others, as *Cedrus Libani*, the bract is a minute flap, and its bundle dies before it reaches the free portion. Between these two extremes are many intermediate forms. That the bract is a modified leaf seems evident at least in some genera. In *Pseudotsuga* and *Larix* there is a gradual transition from ordinary foliage leaves to bracts of a well developed sporophyll; and in abnormal cones of *Picea*, *Larix*, etc., the bracts are like the vegetative leaves. The scale is well developed in all the Abietineae.

The vascular anatomy of the megasporophyll is less variable in the different genera of the Abietineae than is the case in any of the other five groups.

In the lower one-third or more of the strobilus of *Pinus maritima* and *P. Banksiana* there is a general sterilization, beginning with failure of the ovules to produce seed, followed lower down by the abortion of the ovules, and finally at the base of the strobilus the reduction in size and final disappearance of bract or scale or both. In *P. Banksiana* the bract disappears before the scale, but both are finally lost, and between the lowest ovuliferous scales and the bud scales is a region where the strobilus stalk is smooth except for slight elevations (fig. 2). Each of these elevations is supplied with a small vascular strand and suggests a vestige of a megasporophyll. In *P. maritima* only the scale suffers reduction and loss, and the bract, reduced throughout the strobilus, increases in size toward the base (fig. 1).

Correlative with the sterilization and reduction of the appendages in the lower portion of the strobilus are variations in mode of origin of their vascular supplies (figs. 3-29). In the upper half of the strobilus the bract supply originates as a single bundle at the base of the cylinder gap. The scale originates as three or four bundles instead of two as in other Abietineae, one at each side of the

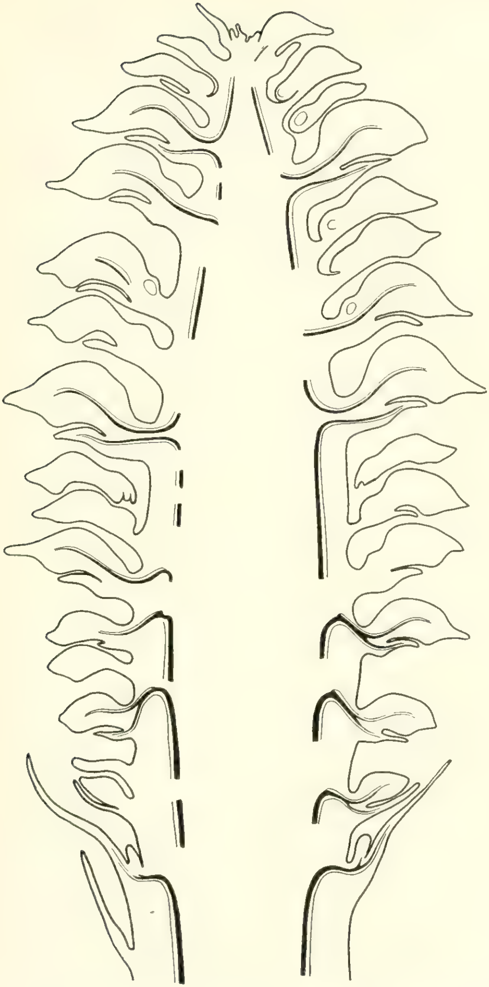


FIG. 1.—*Pinus maritima:* longitudinal section of ovulate strobilus; vascular supplies to bract and scale of each sporophyll, separated in cortex of strobilus by mass of parenchyma in upper portion, approach one another near middle and merge in lower portion of strobilus; in lower portion of strobilus scale decreases in size, while bract becomes comparatively larger.

gap some distance above the bract bundle, and above these again one at each side of gap, or one at one side only. The two uppermost

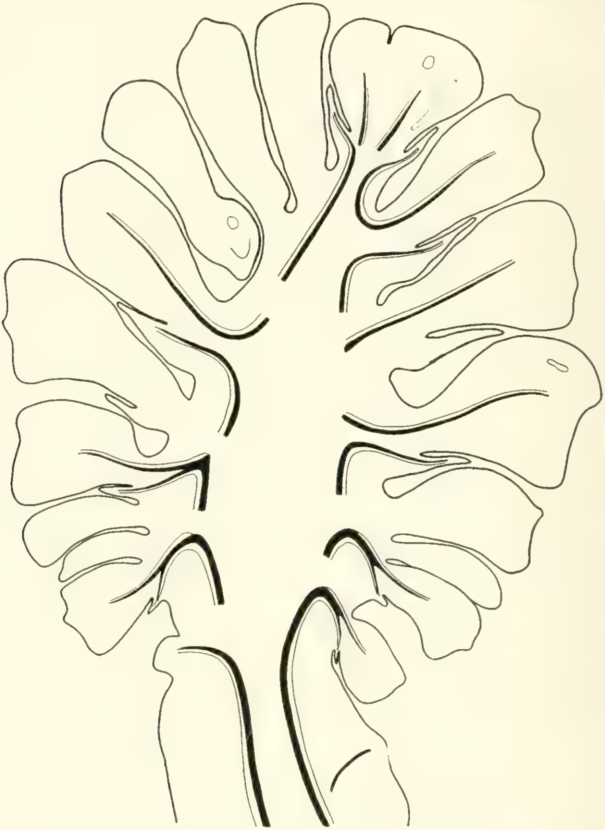
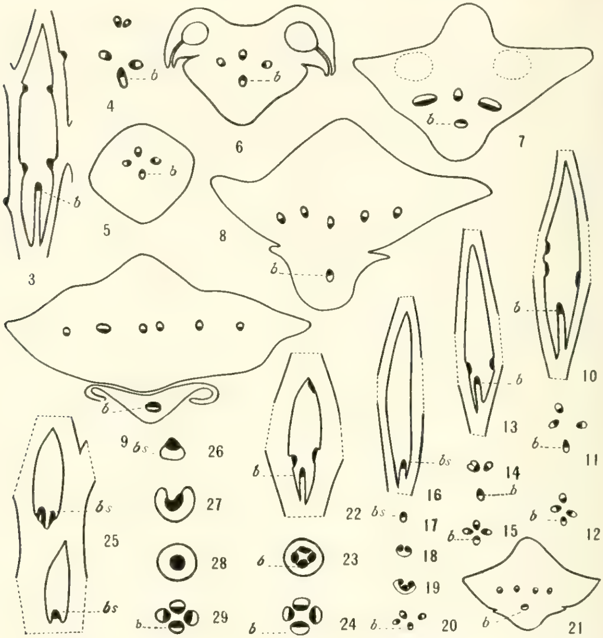


FIG. 2.—*Pinus Banksiana:* longitudinal section of ovulate strobilus; bract and scale supplies, as in *P. maritima*, separated in upper region of strobilus but become merged in lower; unlike *P. maritima*, bract becomes very small in lowest sporophylls; beneath lowest sporophylls are humps, each supplied with a bundle.

bundles soon unite into one, so that four bundles in all with xylem facing result; the lower passes undivided to the end of the bract, the three remaining spread out and give rise in the scale to a row of inverted bundles, seven or more in *P. maritima*, twelve or more in *P. Banksiana*. No branches are seen to bend toward either of the two inverted ovules. Below the middle of the strobilus the uppermost one or two bundles, as the case may be, originate near the lower scale bundles or become united with the latter so that the scale supply starts as two bundles. One of the two bundles soon divides, so that four bundles including that of the bract result; the subsequent course in the appendages is as described before. In the lower sporophyll, the scale supply originates as one or two bundles very near the bract bundle. In the lowest sporophylls the bract and scale supply originates as a single bundle at the base of the gap. In either case there is a subsequent separation into four bundles which supply their respective appendages as before described. In this region of the strobilus the four bundles remain closer together in their course through the cortex than is the case in the upper. In *P. Banksiana* the phloem is continuous around the four xylem strands, which except for a few parenchyma cells would form a solid xylem strand. Near the base of the strobilus, in this species, where the sporophyll supply begins as a single bundle, the xylem creeps around its protoxylem as a pivot and the phloem about the xylem, so that a concentric bundle results. On nearing the appendage the bundle drops into four. In this species the gaps are small, owing to a shortening of the strobilus axis. In both species the sporophyll supply takes a diagonal downward course through the cortex in the lower part of the strobilus, in contrast to the diagonal upward course taken near the tip. This is also likely due to a shortening of the strobilus axis and a consequent crowding of the appendages.

For the sake of comparison, the anatomy of a young vegetative shoot was investigated. In *P. maritima* and *P. Banksiana* (figs. 30–35), whether the bud is to give rise to a spur shoot or a long branch, a single bundle springs from the base of the cylinder gap and supplies the bract, while two other bundles, one at each side of the gap, supply the bud. The bud bundles increase in size and divide, giving rise to a semicircle of bundles with xylem on the concave side, facing the xylem of the bract bundle. The semicircle



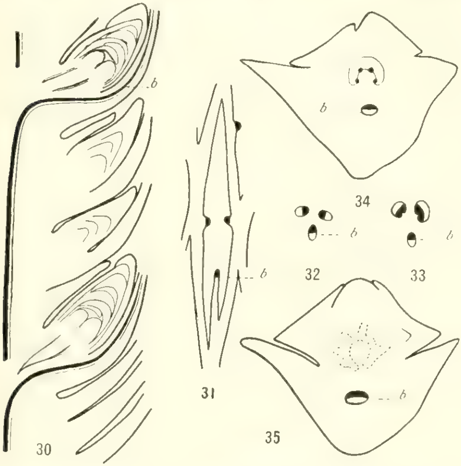
FIGS. 3–21.—*Pinus maritima:* figs. 3–9, course of bundles to bract and scale in upper half of strobilus; fig. 3, origin of bract bundle (*b*) and scale bundles (4 remaining) in strobilus cylinder; fig. 4, two upper bundles uniting; figs. 5–9, transverse sections of sporophyll, bract bundle remaining undivided, scale bundles giving rise to several bundles; figs. 10–12 and figs. 13–15, from lower half of strobilus, scale bundles decreasing in number and originating nearer to bract bundle as base of cone is approached; figs. 16–20, from lowest sporophylls, bract and scale bundles (*bs*) one in origin; fig. 21, transverse section of one of lowest sporophylls; ×16.

FIGS. 22–29.—*Pinus Banksiana:* figs. 22–24, from middle of cone, bundles closing up to form concentric cylinder; figs. 25–29, from lower portion of cone, phloem creeps about xylem so that concentric bundle is formed; ×34.

In all cases four bundles result (figs. 5, 12, 15, 20, 24, 29), the lower supplying the bract, the remaining three the scale.

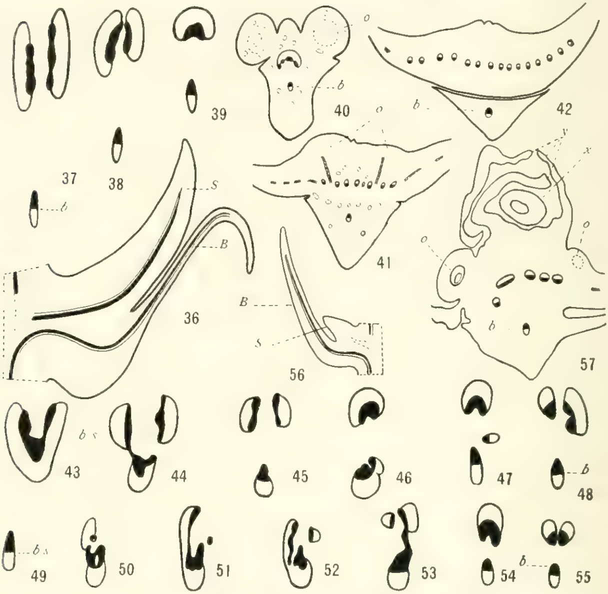
gradually approaches a circle, and in a slightly older bud closes to form the cylinder of the shoot. The bud is united with the bract at its base, and the general appearance of a young bud and its subtending bract is suggestive of a young abietinean sporophyll.

In *Keteleeria Fortunei* (figs. 36–57), one bundle originates near the base of the gap in the strobilus cylinder and supplies the bract. It remains undivided throughout its course. Two bundles, one from each side of the gap, supply the scale. The two bundles soon unite, forming one inverted bundle, that is, its xylem faces the xylem of the bract. The inverted bundle then breaks, forming a semicircle of bundles which spread and divide, forming in the scale a row of sixteen or more bundles. Near the ovular insertion a small concentric branch is given off to each of the two inverted ovules.



FIGS. 30–35.—*Pinus Banksiana*, vegetative bud: fig. 30, radial longitudinal section of lower portion of young vegetative branch; a vegetative bud in axil of each bract except at base of branch; fig. 31, origin of bract (*b*) and bud bundles; figs. 32–35, transverse sections, young bundles of bud beginning to form cylinder of branch; ×16.

In the lower sporophylls the bract and scale bundles originate in close proximity at the base of the gap. In the lowest sporophylls the vascular supplies to both organs originate as one bundle, and the two systems separate later for their respective organs. In some of the lowest sporophylls the scale supply begins blindly in the scale.



FIGS. 36–57.—*Keteleeria Fortunei:* fig. 36, longitudinal section of sporophyll, ×16; figs. 37–42, transverse sections of sporophyll from upper half of strobilus; figs. 36–39, two scale bundles divide actively, a single strand is given off to each ovule (fig. 41, *o*), ×7; figs. 43–48, from one of lower sporophylls, bract and scale bundles, close together in origin (fig. 47), gradually separate, ×34; figs. 49–55, from one of lowest sporophylls, bract and scale bundles united in origin gradually become distinct, ×34; fig. 56, longitudinal section of young sporophyll, scale (*S*) still comparatively small, ×7; fig. 57, transverse section of one of lowest sporophylls showing outgrowths (*x*, *y*) between ovules (*o*), ×16.

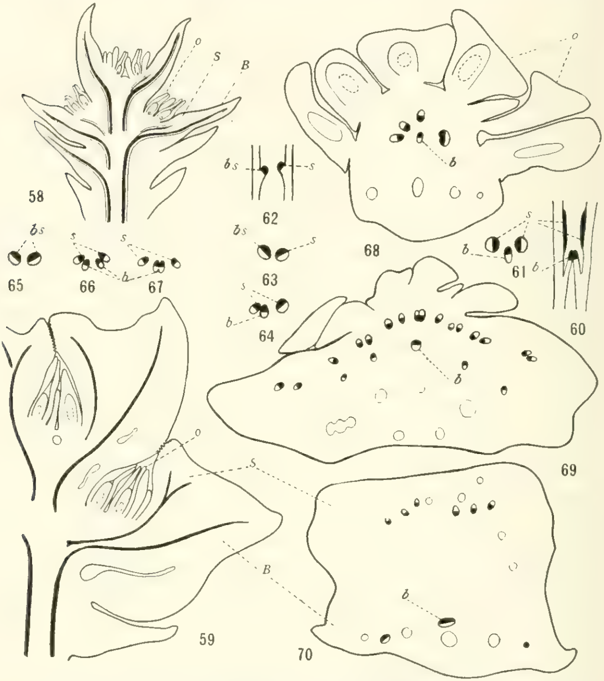
Between the ovules of the lower sporophylls is found an outgrowth which suggests an ovule with a poorly developed nucellus. At each side of and behind this median outgrowth are others which are more bractlike. In some of these xylem cells are present. Whether these abnormal excrescences mean reversions to ancestral features may be difficult to determine. The young strobilus shows no such outgrowths.

Cupressineae

The general features of the ovulate strobilus in the Cupressineae are the great reduction in the number of the sporophylls, the cyclic arrangement of the sporophylls, complete coalescence of bract and scale, and erect ovules variable in number.

The strobilus of *Cupressus Benthamii* (figs. 58–70) is composed of four decussate pairs of sporophylls. Many erect ovules are packed at the base of each sporophyll. At the megaspore mother cell stage the only evidence of the scale is a slight elevation back of the ovules and the differentiation of the scale supply near the strobilus cylinder. In somewhat later stages the free part of the scale projects outward almost as prominently as that of the bract. Contact and interlocking of epidermal cells takes place between neighboring sporophylls in such a way that the ovules become entirely inclosed.

The vascular anatomy is slightly variable in the individual sporophylls of a strobilus. As in other cyclic forms, the cylinder gap fails to close after the departure of the appendage vascular supply, and hence is continuous with the one above and below. The bract supply may arise as a single strand at one side of the continuous gap, or as two, one from each side of the gap, in which latter case the two unite into one. The scale supply originates as two bundles, one from each side some distance above the bract supply. In other cases two bundles, one from each side of the gap, compose both bract and scale supply. A strand may be given off from one of the bundles to form the bract bundle, or a strand may be given off from each bundle and the two strands unite to form the bract bundle. Whatever may be the origin, the bract bundle remains undivided to the tip of the bract. The scale bundles divide actively; the majority come to lie near the dorsal side of the sporophyll and have inverted orientation of xylem and phloem, but several twist around to each side of the bract bundle and have

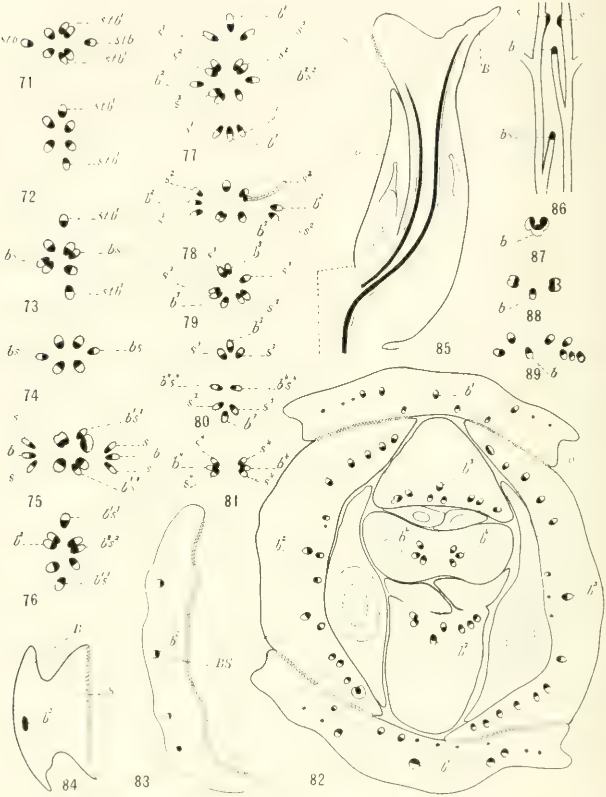


FIGS. 58–70.—*Cupressus Benthamii:* fig. 58, longitudinal section of young strobilus, scale (*S*) evident only by slight elevations on upper side of sporophyll, and beginning of scale bundles; fig. 59, longitudinal section of older strobilus, scale (*S*) evident but incorporated with tissues of bract (*B*), ×7; figs. 60–67, three methods of origin of sporophyll bundles; figs. 60, 61, bract (*b*) and scale bundles (*s*) distinct in cylinder gap; figs. 62–64, bract bundle derived from one of scale bundles (*bs*); figs. 65–67, bract bundle derived from both scale bundles (*bs*); figs. 68–70, transverse sections of sporophyll, scale bundles divide vigorously, some scale bundles persist in bract portion (fig. 70, *B*), ×16; *o*, ovule.

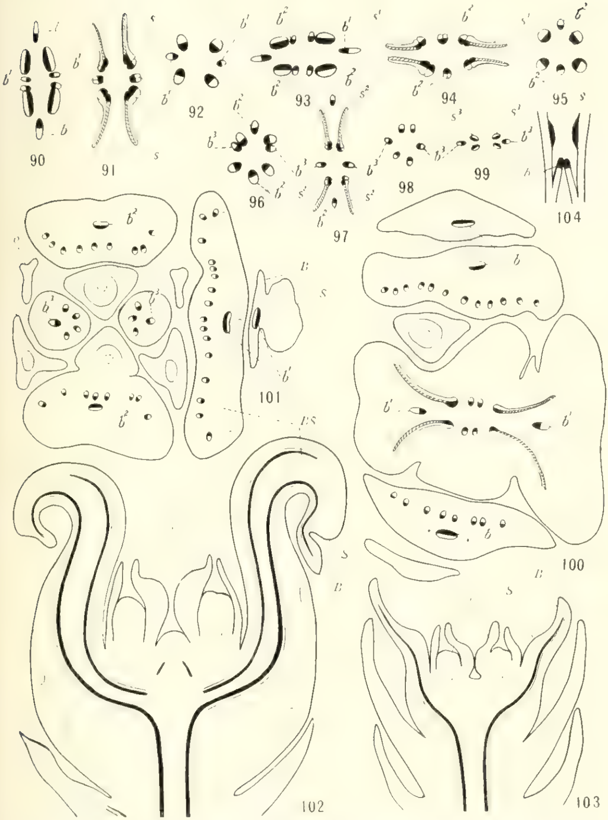
the same orientation of parts as the latter bundle. A fact to be noted in the further course of the bundles is that some of the lower scale bundles extend into the bract, though not as far as the bract bundle.

The ovulate strobilus of *Thuja occidentalis* (figs. 71 89) consists of about five decussate pairs of sporophylls. The two lower pairs are sterile, the third may have only one ovule to each sporophyll, the fourth two ovules to the sporophyll, and the fifth is sterile. The tissues of the bract and scale are so closely welded that it is impossible to distinguish one from the other except at the very tip. In the upper fertile pair of sporophylls the vascular supplies to bract and scale are distinct in origin. The supply of the first arises as one bundle at one side of the continuous gap; that of the latter as two bundles, one at each side and at a higher level. The bract bundle does not divide; the scale bundles give rise to numerous bundles, a few of which become inverted and lie near the dorsal side of the sporophyll; the majority, together with the bract bundle, form an irregular lower row of inverted bundles. In the two sterile pairs at the base, the bract and scale supply have their beginning as one bundle at one side of the long gap. This bundle soon drops into three, the median one supplying the bract, the two lateral the scale. The further course is as described before for the upper fertile sporophyll. In the lower fertile sporophyll is found a combination of the two methods of bundle origin mentioned. One of the scale bundles rises separately, but the other is combined with the bract bundle and rises at a lower level; it later separates from the bract bundle. The sterile tip pair of sporophylls receives the last two bundles of the strobilus axis. One bundle goes to each sporophyll, and drops into three bundles which divide further.

The strobilus of *Chamaecyparis Lawsoniana* (figs. 90–104) consists of about four pairs of sporophylls. In the cone represented in cross-section (figs. 100, 101), one of the lowest sporophylls has one lateral ovule, each of the sporophylls of the second pair two lateral ovules, and each of the third pair one median ovule; the fourth pair is sterile. In the early free-nuclear stage the scale is apparent only as a slight elevation on the dorsal side of the bract, which at this stage is almost straight. At a little later stage



FIGS. 71–89.—*Thuja occidentalis:* figs. 71–81, transverse section of strobilus axis, showing origin of sterile bract bundles (*stb*, stb^1) below cone, bract bundles (b-b^4) of sporophylls, and scale bundles (s-s^4); fig. 82, transverse section of same strobilus, many of scale bundles become oriented like bract bundle, two pairs of sporophylls fertile, one pair bearing one ovule per sporophyll, the other two ovules; figs. 83, 84, transverse sections near tip of sporophyll, bract (*B*) and scale (*S*) beginning to separate; fig. 85, longitudinal section of sporophyll, bract (*B*) and scale (*S*) closely united; figs. 86–89, sections parallel to cone axis; fig. 86, bract (*b*) and scale (*s*) bundles distinct in origin in upper part of strobilus, united in lower (*bs*); figs. 87–89, bract bundle separating from scale bundles; ×16.



FIGS. 90–104.—*Chamaecyparis Lawsoniana:* figs. 90–99, transverse sections of strobilus axis, showing origin of bract (b–b^3) and scale (s–s^3) bundles; figs. 100, 101, transverse sections of same strobilus, scale bundles remain close to dorsal side; one of sporophylls of lowest pair bears one lateral ovule, each of second pair two lateral ovules, each of third one median ovule, fourth pair sterile; fig. 102, longitudinal section of strobilus, bract (B) and scale (S) separate only at recurved tip of sporophyll; fig. 103, longitudinal section of younger strobilus, scale (S) evident as slight elevation on dorsal side; fig. 104, origin of bract (b) and scale bundles (s) in cylinder gap; ×16.

the growth on the dorsal side of the sporophyll is very marked, resulting in the deflexing of the coalesced bract and scale. The bract is free only at the tip of the deflexed portion. The vascular systems to the bract and scale are distinct from the strobilus cylinder. The bract bundle remains single to the tip of the bract. The two scale bundles end in a dorsal row of numerous inverted bundles. The flank bundle may turn partly and lie nearer the ventral side of the appendage.

The strobilus of *Juniperus communis* (figs. 105–113) consists of a whorl of three sporophylls united at the base and surrounding three ovules. In the older stage the sporophylls coalesce also at the tip, forming a fleshy berry-like body. The coalescence of bract and scale is complete.

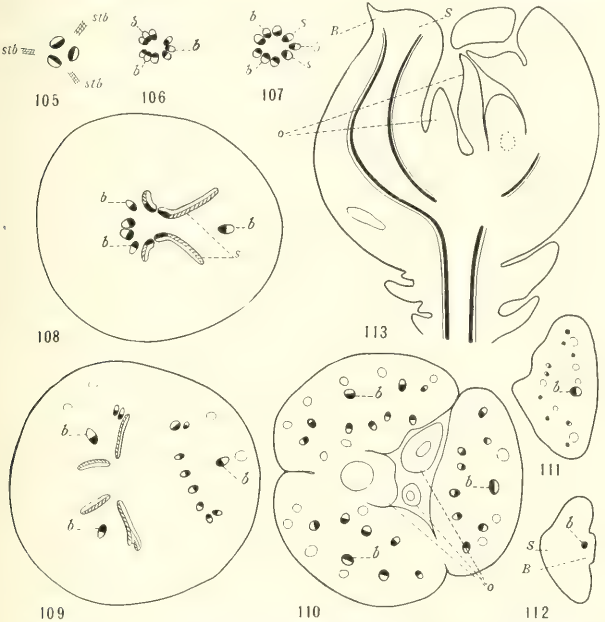
After the traces to the three bracts beneath the sporophylls have left the cylinder, each of the three remaining bundles divides into three; the median or bract bundle proceeds undivided into the bract; the two lateral or scale bundles divide, giving off some bundles to the dorsal side of sporophyll and some to ventral in such way that, including bract bundle, an oval ring of bundles with xylem facing is formed.

TAXODINEAE

The general features of the ovulate strobilus in the Taxodineae are spiral arrangement of sporophylls, reduction in number of sporophylls in some forms, considerable coalescence of bract and scale, and varying number and orientation of ovules.

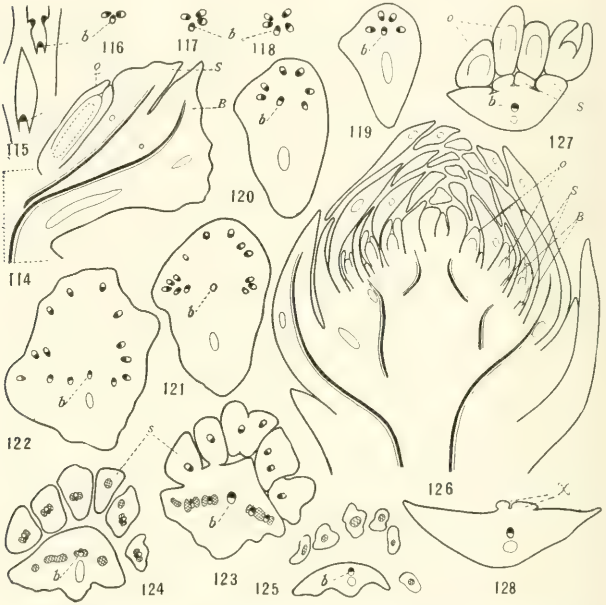
In *Cryptomeria japonica* (figs. 114–128) the axis of the young strobilus is very short and the sporophylls are crowded at the broadened summit. The ovules at this stage appear to be inserted on the strobilus axis. The scale begins its development as four to six lobes between the ovules and the dorsal side of the bract. In an older stage the axis has elongated, forming a globose cone. The erect ovules, usually three in number, are definitely inserted on the sporophyll. The lobed scale is united for two-thirds of its length to the bract. In the upper portion of the cone three bundles leave the axis for the sporophyll; one at the base of the gap to supply the bract, and one from each side of gap to supply the scale.

The two scale bundles divide, resulting in the sporophyll in a ring of bundles with xylem facing inward. Included in the lower portion of the ring is the bract bundle. One and sometimes two bundles enter each lobe of the scale. A number of the scale bundles



FIGS. 105–113.—*Juniperus communis:* figs. 105–110, course of bundles from base of strobilus to middle of three sporophylls; sporophylls closely coalesced at base; some of scale bundles come to lie in same plane as bract bundle (*b*); figs. 111, 112, transverse sections of tip of sporophyll, bract apparent as small ridge (*B*); fig. 113, longitudinal section of strobilus; *o*, ovule; *stb*, sterile bracts below strobilus; ×16.

pass into the free portion of the bract at each side of the bract bundle and have the same orientation of parts as the bract bundle. The ending of each bundle is accompanied by short irregular tracheids. The scale bundle may arise in closer proximity to the bract bundle; in the lower sporophylls scale and bract supplies spring from the base of the gap as one large bundle which breaks into three; these latter supply the sporophyll parts as described before.



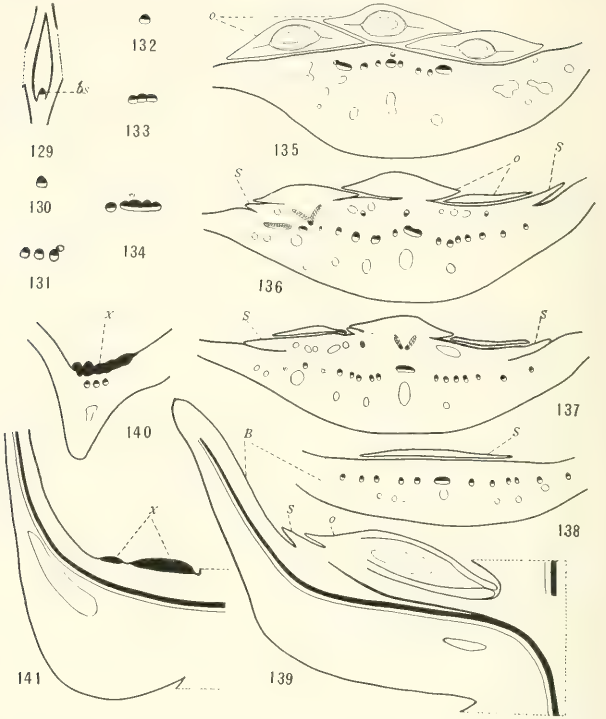
FIGS. 114–128.—*Cryptomeria japonica:* fig. 114, longitudinal section of sporophyll; fig. 115, origin of bract (*b*) and scale bundles in upper part of strobilus distinct, in lower part united (*bs*); figs. 116–125, transverse sections, further course of bundles in sporophyll; bract bundle (*b*) remains undivided, some of scale bundles come to lie in same plane as bract bundle (figs. 121, 122); lobes of scale separate from one another and from bract, each lobe receiving usually one bundle; remaining scale bundles enter free portion of bract (figs. 123, 124); bundles end in large irregular tracheids; ovules not represented; ×7; fig. 126, longitudinal section of young strobilus, scale (*S*) appears as small lobes between bract (*B*) and ovules (*o*), ×10; fig. 127, transverse section of young sporophyll, showing bract (*B*), four lobes of scale (*S*), and ovules (*o*); fig. 128, transverse section of one of uppermost sterile sporophylls, showing outgrowths similar to lobes of scale in fig. 127, ×34.

The strobilus of *Taxodium distichum* possesses the same general characters as described for *Cryptomeria japonica.*

The strobilus of *Cunninghamia Davidiana* (figs. 129-141) is composed of a relatively large number of sporophylls. The scale is united to the bract, with the exception of a small edge distal to the insertion of the three inverted and slightly winged ovules. The vascular supply to the sporophyll departs from the cylinder gap as one large bundle; this bundle soon divides more or less definitely into three; a further division takes place until a row of fifteen or more normally oriented bundles results in the expanded part of the sporophyll. In the earlier course of the branching of the bundles weak strands separate from the lateral bundles and swing around 180° so as to lie on the dorsal side of sporophyll; some of these strands fork, so that an upper row of five or six inverted bundles results; some of these bundles may begin blindly; near the insertion of the ovules one or two strands bend toward the chalaza and end there or a short distance behind the chalaza. Owing to the fact that the bundles in the lower row adhere more or less in the earlier course of division, it is difficult to determine whether the median of the three first bundles passes undivided into the narrow portion of the bract. It is accompanied, however, by branches from the lateral bundles for some distance into the free portion of the bract. At the tip and base of the strobilus are sterile sporophylls. These have a vascular anatomy similar to that of the fertile sporophylls, with the exception of the absence of the upper inverted bundles in the former. Neither scale nor ovules are present, but in place of these appear slight excrescences with different staining reactions.

ARAUCARINEAE

The ovulate strobilus in the Araucarineae is composed of numerous spirally arranged and closely compacted sporophylls. Each sporophyll bears one inverted ovule, which is imbedded in the sporophyll tissues in *Araucaria*, and naked and winged on one side in *Agathis*. Another feature of the Araucarineae of significance in this connection is the branching of the sporophyll bundle in the



FIGS. 129–141.—*Cunninghamia Davidiana:* fig. 129, vascular supplies to bract and scale leave cylinder gap as one bundle (*bs*); figs. 130, 131, and 132–134, splitting up of single bundle; figs. 135–138, transverse sections of sporophyll small strands split off from lower series or begin blindly (fig. 134, dots) and come to lie on dorsal side of sporophyll; the majority of these small bundles finally bend toward chalaza of ovules and end there; the majority of lower bundles enter free portion of bract (fig. 138, *B*) beyond insertion of scale (*S*); fig. 139, longitudinal section of sporophyll, scale (*S*) appears as flap beyond insertion of ovules (*o*); fig. 140, transverse section of sterile sporophyll near tip of strobilus, dark stained elevations (*x*) taking place of scale and ovules; fig. 141, longitudinal section of sterile sporophyll at base of cone, similar outgrowths (*x*) as in fig. 140; ×7.

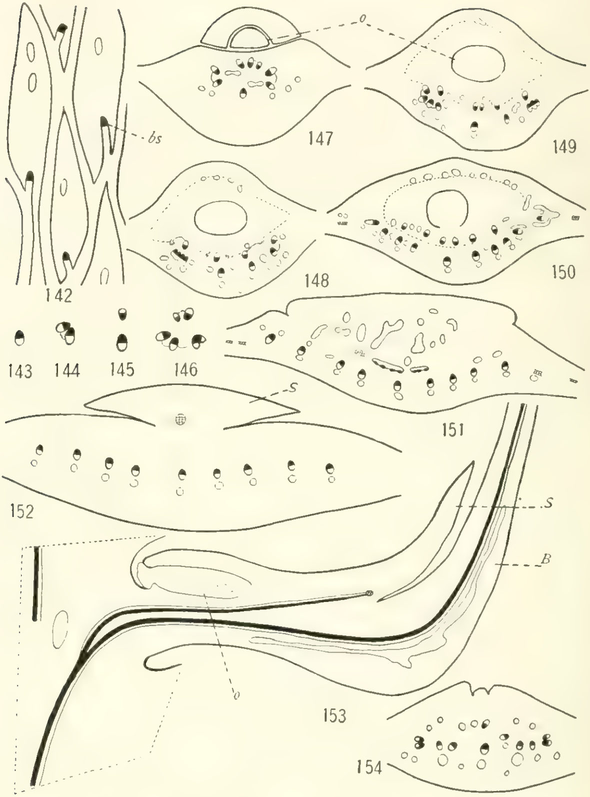
vegetative leaf. This is a characteristic which so far as investigated occurs outside the Araucarineae only in *Podocarpus Nageia*.

The sporophyll of *Araucaria* is characterized by the so-called ligule, which there is good reason to believe is homologous with the scale described in the foregoing groups. In the young strobilus of *Araucaria Rulei* and *Araucaria Balansi* the comparatively small ligule is attached to the bract at its base only, the greater portion being free. In the older strobilus it is free only at the tip. Distal to the line of coalescence of bract and scale the bract remains thick and wide, then becomes narrow and stiff, resembling the bracts at the base of the strobilus.

The vascular supply to the sporophyll of *A. Balansi* (figs. 142–154) arises as a single bundle near the base of the gap in the strobilus cylinder. In the middle of the cortex the single bundle divides into two unequal parts, the smaller of which twists through an angle of 180° so as to lie above the parent bundle with its xylem facing the xylem of the latter. The upper bundle may or may not divide at this stage; the lower bundle divides into two or three; in case of two, one bundle soon divides and a median lower bundle is formed. The lateral bundles divide actively; some of the resulting bundles become inverted and lie on a level with the upper bundle, others normally oriented come to lie on a level with the lower median bundle. Where the sporophyll broadens, branches with normal orientation enter the wings. Behind the ovules the bundles of the upper series begin to converge in groups, and finally end in masses of irregular tracheids. A bundle is sometimes found to continue almost to the free portion of the ligule. Where the bract becomes narrow, the lower bundles also end, with the exception of about three median ones which extend into the slender portion. In the lowest sporophylls, where the ovule and scale are poorly developed or absent, the upper bundles are weak and few in number, or wanting.

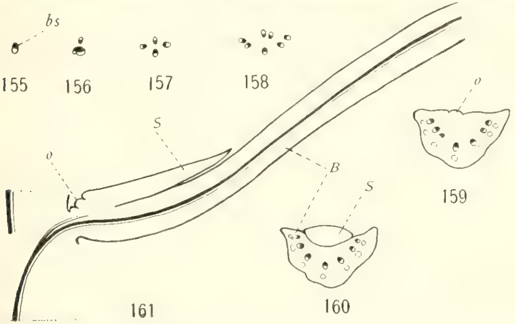
In the other forms investigated, *A. Rulei* (figs. 155–161) and *A. excelsa*, the general features are as in the above-described species.

The strobilus of *Agathis australis* (figs. 162–170) is composed of numerous sporophylls which are very closely packed, probably owing to the shortening of the strobilus axis. A ligule, as found in



FIGS. 142–154.—*Araucaria Balansi:* fig. 142, vascular supply to sporophyll springs from single strand near base of cylinder gap; figs. 143–146, course of bundles in cortex; figs. 147–152, transverse sections of sporophyll, lines drawn between bundles indicate last division; upper bundles run together in groups and end in large irregular tracheids (fig. 151); one persists almost to free portion of scale (fig. 152); fig. 153, longitudinal section of sporophyll (*S*, scale or ligule; *B*, bract, *o*, ovule); fig. 154, transverse section of sterile sporophyll at base of strobilus, upper bundles few; ×8.5.

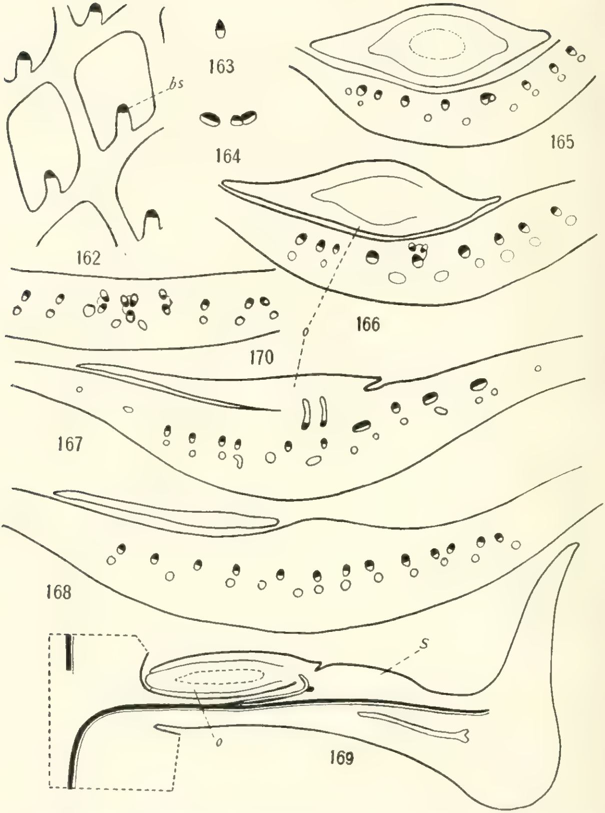
Araucaria, is absent, but the general appearance of a slight elevation behind the ovule suggests the possibility of a fused body. The vascular supply begins as a single stout trace a little to one side at the base of the short wide cylinder gap. The trace increases in size, and in the outer one-third of the cortex a branching begins which in the lamina of the scale results in about thirteen bundles. About half-way between the insertion of the sporophyll and the insertion of the ovule a small strand splits off from one of the median bundles, swings around 180°, and becomes an inverted bundle opposite its sister bundle. The inverted strand may fork, and also inverted strands may spring from several of the median lower bundles and these strands may divide so that the number of upper bundles varies from one to several. In any case, each of the upper bundles, after giving off a phloem-like strand to each ovule, ends in large irregular tracheids.



FIGS. 155–161.—*Araucaria Rulei*, young sporophyll: figs. 155–160, course of bundles from strobilus axis to free portion of bract, upper bundles differentiated in cortex and base of sporophyll (*B*, bract; *S*, scale; *o*, ovule); fig. 161, longitudinal section of sporophyll; ×8.5.

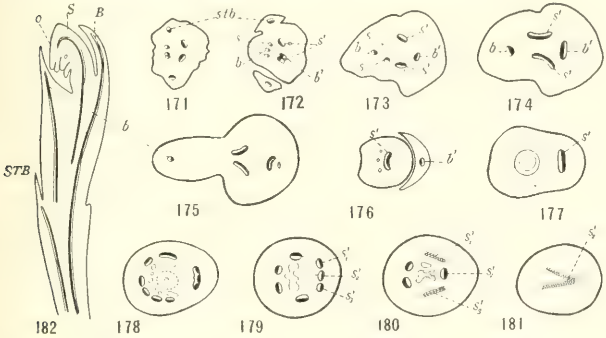
PODOCARPINEAE

In most of the Podocarpineae a definite strobilus is absent, the fructification consisting in most cases of one or two fertile sporophylls. In many forms there is a tendency for some part of the fructification to mature fleshy.



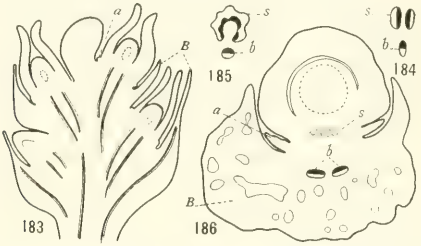
Figs. 162–170.—*Agathis australis:* fig. 162, vascular supply to sporophyll springs from single strand near base of cylinder gap; figs. 163–168, course of bundles in cortex and sporophyll, a bundle springs from a median bundle, becomes inverted (fig. 166), then breaks into two branches which enter chalaza of ovule (fig. 168), ×8.5; fig. 169, longitudinal section of sporophyll, an elevation appears distal to insertion of ovule; fig. 170, transverse section of sporophyll, inverted bundles have sprung from three of median lower.

In a species of the subgenus *Eupodocarpus* (figs. 171–182) the ovule-bearing branch is a dwarf lateral branch bearing at its tip one and occasionally two fertile sporophylls, and a few small bracts some distance below. The single inverted ovule is imbedded in the tissues of the "epimatium," which there is evidence for believing to be homologous with the ovuliferous scale in other conifers. At maturity the portion of the fruiting branch between the fertile sporophylls and the upper sterile bracts becomes fleshy.



FIGS. 171–182.—*Podocarpus* (sp. of *Eupodocarpus*): figs. 171–181, transverse sections from base of uppermost sterile bracts (fig. 182, *STB*) to tip of strobilus; *stb*, bundles supplying sterile pair of bracts; *b*, bundle supplying sterile bract opposite fertile sporophyll; *s*, bundles inclining toward sterile bract then ending; b^1, bundle supplying bract of fertile sporophyll; s^1, two bundles uniting into one enter scale of fertile sporophyll where the single bundle breaks into three (fig. 179, s^1_1, s^1_2, s^1_3); distal to chalaza each of three bundles bends to pass down dorsal side of ovule (figs. 180, 181; fig. 170; dotted lines between bundles indicate that one bundle is a continuation of the other); fig. 182, longitudinal section of strobilus; ×7.

The vascular anatomy was investigated only in cases of one fertile sporophyll. The vascular supply of the branch axis, after the traces to the sterile bracts have gone out, consists of three small and three large bundles. The median of the three smaller bundles enters what in the young stages appears like a bract opposite the fertile sporophyll; the two small lateral bundles bend in the same direction as the small median bundle, then end. The median of the three larger bundles supplies the bract of the fertile sporophyll; each of the lateral bundles twists so as to lie with xylem facing the bract bundle, then the two unite into one bundle which proceeds into the scale. The single bundle increases in size, and on nearing the chalaza of the ovule breaks into three; at the base of the ovule each of the lateral branches curves over and passes downward in the tissues of the scale flanking the ovule. The median bundle proceeds slightly farther, then curves over the base of the ovule, and at the same time breaks into two branches which pass downward in the portion of the scale on the dorsal side of the ovule. The recurved branches of the scale supply fork so that in cross-section a ring of bundles with xylem facing outward surrounds the ovule.



FIGS. 183–186.—*Phyllocladus alpinus:* fig. 183, longitudinal section of young strobilus, aril (*a*) appearing around the base of the ovule; figs. 184, 185, scale (*s*) bundles unite to form a semicircle; fig. 186, transverse section of an older sporophyll, bract bundle (*b*) forks in this instance, scale bundle (*s*) ends at base of ovule; ×61.

In another species of *Podocarpus* investigated the scale bundle divides into two instead of three branches. Each of these two curves at the chalazal end of the ovule and passes downward, one at each side of the ovule. The crests of the two curves are connected by irregular xylem cells, and a strand of similar tissue extends from each crest outward into the protuberance on the scale behind the ovule.

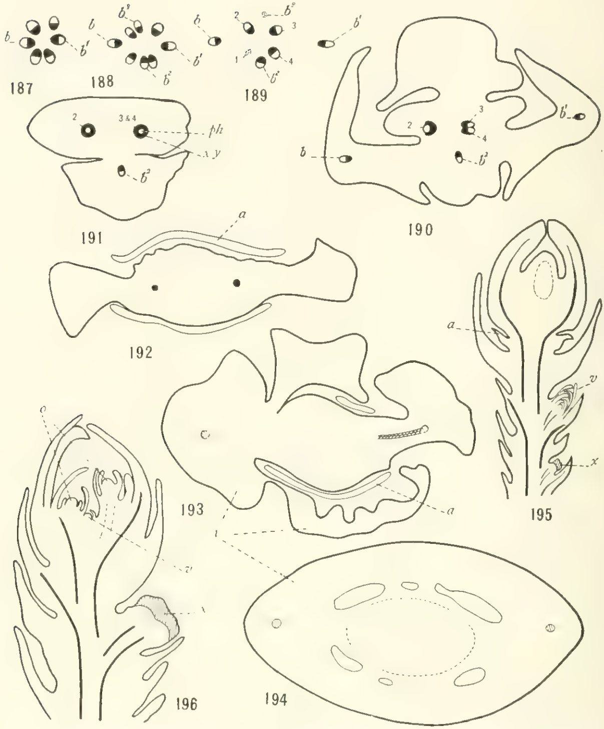
Phyllocladus alpinus (figs. 183–186) consists of a globose strobilus of few sporophylls. The ovule is sessile in the axil of the bract. The vascular supply to the bract springs from the base of the gap. Two bundles from the sides of the gap unite to form a semicircle which ends at the base of the ovule.

TAXINEAE

Perhaps the most outstanding features of the Taxineae in this connection are the distinctly terminal ovule in some forms and the development of the aril in the later stages.

In *Taxus baccata* (figs. 187–196) the bud of the primary fertile and dwarf branch develops in the axil of a leaf of a long branch of the first season's growth; the second season the ovule matures. Near the tip of the primary fertile and dwarf branch is a secondary dwarf branch bearing a few decussate pairs of bracts and terminally an ovule. Occasionally two secondary dwarf ovule-bearing branches are produced, one at each side of the terminal vegetative bud of the primary branch. Beneath the ovuliferous branch or branches the primary shoot is covered with scalelike bracts. The growth of the terminal vegetative bud of the primary shoot is usually arrested by the active development of the secondary ovuliferous branch, so that the latter appears to be terminal. Ovules are found maturing on the second, third, and fourth seasons' growth, instead of on the second season's only. This implies that the ovule in some cases fails to mature the second season, or that the vegetative bud of the primary dwarf branch may resume activity and produce other ovuliferous branches; the latter seems most probable; also the presence of branch scars on the older and more elongated dwarf shoots suggests that the latter conclusion may be drawn.

After the bundles to the upper sterile bracts have either passed into their respective appendages or have ended before entering the latter, the vascular supply of the axis of the ovule-producing branch consists of four bundles. These bundles unite in pairs, one bundle from each side of the bract bundle of the next lower pair, and not one from each side of the bract of the last pair as is usually the case where the united bundle is to supply some axillary structure. The xylem creeps around the phloem in each of the two bundles formed. Sometimes one of the four bundles in the axis ends, whereupon the odd bundle behaves like the fused bundle



FIGS. 187–196.—*Taxus baccata:* figs. 187–194, transverse sections upper part of fertile branch to middle or ovule; b, b^1, bundles to supply pair of bracts; b^2, b^3, bundles to supply uppermost pair of bracts; b^3 ends before entering bract; 1, ends in axis; 2, xylem surrounds phloem; 3, 4, unite into one bundle, xylem surrounds phloem (fig. 191), the two bundles continue as weak strands of xylem cells in base of each wing of ovule, xylem cells are soon replaced by elongated slightly thickened cells without true xylem markings, ×16; fig. 105, longitudinal section of fertile branch, terminal vegetative bud (v) is turned aside by rapidly growing ovule bearing branch; x, scar left by vegetative or fertile bud or branch, ×7; fig. 106, young fertile branch showing two lateral ovule-bearing branches and a terminal vegetative bud (v); x, scar as in fig. 105, ×16.

(figs. 183, 186). At the base of the ovule each bundle passes into a mass of short irregular tracheids of large caliber. From each mass arises a small strand consisting of poorly developed xylem soon replaced by elongated thickened cells without xylem markings. Each strand passes upward in the thickened ridge of the ovule.

Discussion

Assuming that the megasporophyll in all the forms is a composite organ, consisting of bract and axillary scale, the investigated forms may be divided into four general groups based on the relation of bract and scale to one another.

In the first group the bract and scale are separate almost to the base of the appendage and both are about equally prominent. Here belong *Keteleeria* (fig. 36), *Pseudotsuga*, species of *Abies*, and species of *Larix*.

In the second group the bract and scale are separate as in the first group, but the bract, at least in the later stages, is much less prominent than the scale, and in certain instances appears distinctly to be on its way to obliteration. In this group belong species of *Abies* (**2**), species of *Larix*, *Tsuga*, *Picea*, *Pinus* (figs. 1, 2), and species of *Podocarpus* and *Dacrydium* (**4**); *Cedrus Libani* (**2**) and the lower sporophylls of *Pinus Banksiana* (fig. 2) show the bract in process of extinction. In species of *Podocarpus* the scale has folded toward its dorsal side, thus forming the second integument or epimatium of the inverted ovule (fig. 182).

In the third group the bract and scale are considerably to completely welded, but the fused structure shows some evidence of its double nature. Within this group are *Sciadopitys* (**2**), *Sequoia* (**2**), *Cunninghamia* (fig. 139), species of *Arthrotaxis* (**1**), *Cryptomeria* (fig. 114), *Taxodium*, *Thuja* (fig. 85), *Cupressus* (fig. 59), *Chamaecyparis* (fig. 102), *Juniperus* (fig. 113), *Araucaria* (fig. 153), and *Podocarpus dacrydioides* (**4**). In young strobili of *Araucaria Rulei* (fig. 161) and *Cryptomeria japonica* (fig. 126) the bract and scale are distinct almost to the base, and the fused portion becomes comparatively large in the subsequent development of the organ. In *Cupressus Benthamii* (fig. 58), *Thuja occidentalis*, *Chamaecyparis Lawsoniana* (fig. 103), and *Juniperus communis* the scale in the young strobilus is one with the bract and becomes evident in the later stages by the comparatively rapid growth of the tissues on the dorsal side of the composite organ.

In the fourth group there is no external evidence of more than one organ. Into this group fall *Arthrotaxis selaginoides* (**1**), *Agathis* (fig. 169), *Saxegothaea* (**5, 8**), *Phyllocladus* (fig. 183), *Taxus* (fig. 191), *Torreya* (**6, 7**), and *Cephalotaxus* (**6, 7**). It should not be surprising to find forms in which the welding has taken place beyond the recognition of more than a single structure when one considers to what extent this process has taken place in *Chamaecyparis*, *Juniperus*, *Thuja*, *Cunninghamia*, and *Podocarpus dacrydioides* (**4**). The low cushion behind the ovule in *Agathis australis* suggests the complete fusion of a scale to a large bract; a similar fusion is nearing its completion in *Cunninghamia Davidiana*.

On the basis of vascular anatomy the investigated sporophylls fall into two general groups.

In the first group the bract and scale supply arises as separate bundles in the cylinder gap. In this group belong in general those forms in which the two sporophyll parts are separate and fairly well developed, as the seed-producing sporophylls of *Pinus Keteleeria*, *Picea*, *Larix*, *Tsuga*, *Pseudotsuga*, and *Abies* (**2**). To this group belong also many in which the two sporophyll members present considerable to complete fusion, as *Araucaria Bidwilli* (**1, 3, 10**), *Chamaecyparis Lawsoniana*, *Juniperus communis*, and the upper sporophylls of *Thuja occidentalis*, *Cupressus Benthamii*, and *Cryptomeria japonica*. This group includes also some in which the sporophyll is evident only as a single organ, namely *Phyllocladus* and *Cephalotaxus* (**6, 7**).

In *Podocarpus* and *Dacrydium*, where the strobilus consists of one or two sporophylls, and in *Juniperus communis*, the sporophylls receive the final bundles of the axis. There is in these instances no cylinder gap, and the bract and scale supplies, at least in the forms investigated by the writer, result from the division of one of the final bundles in the axis. The early division of the bundle in the tip of the axis perhaps justifies the placing of these forms in this group.

In the second group based on vascular anatomy the bract and scale vascular supplies are more or less intimately united into one bundle which springs from the base of the cylinder gap.

Of sporophylls with parts separate there belong here the lower sporophylls of *Pinus Banksiana*, *P. maritima*, and *Keteleeria Fortunei* (the ovules in these sporophylls are to greater or less extent abortive), and *Cedrus Libani* (**2**). Of the sporophylls with parts considerably united there fall into this group those of *Cunninghamia*, *Arthrotaxis laxifolia* (**1**), most species of *Araucaria* (**1, 3, 6, 7, 9, 10**), and the lower sporophylls of *Cryptomeria japonica*, *Cupressus Benthamii*, and *Thuja occidentalis*. Most of the apparently simple sporophylls are included in this group, as those of *Agathis*, *Saxegothaea* (**5, 8**), and *Arthrotaxis selaginoides* (**1**).

The degree of welding of the bract and scale vascular supplies varies considerably. In *Arthrotaxis selaginoides*, *Agathis*, *Araucaria*, and *Saxegothaea* the two remain united into one bundle for greater or less distance in the cortex; in most of the others the single bundle divides early; but in many cases, where the two sporophyll parts have fused extensively, branches of the scale supply swing about to lie on the ventral side of the appendage at each side of the bract bundle. This fact is well illustrated in *Thuja occidentalis*, *Juniperus communis*, *Cupressus Benthamii*, and *Cryptomeria japonica*. In *Cupressus Benthamii* and *Cryptomeria japonica* the scale bundles at either side of the bract bundle even accompany the bract bundle into the free portion of the bract.

Bundle distribution is generally directly related to the size of the organ supplied, hence the bundles extend into the most expanded region of the sporophyll, whether that particular region represents bract or scale.

In *Cunninghamia*, *Araucaria*, and *Agathis*, in which absence of sporophylls with separate bract and scale supplies makes comparison impossible, it is difficult to determine with certainty what is bract and what is scale supply. The matter is further complicated by the presence in the last two genera of a branching bundle in the vegetative leaf, a condition which probably implies a branching bract bundle in the bract of the sporophyll as well. And, further, *Cupressus Benthamii* and *Cryptomeria* have clearly shown that the final destination of a bundle does not always determine to which sporophyll part it belongs. It is to be suspected that the bundle system of *Agathis* and *Araucaria* represents a complex of bract and scale bundles. Judging by the course of events in other conifers with single-veined vegetative leaves, it may be suggested that the large median lower bundle in *Cunninghamia Davidiana* is the bract bundle proper, which is accompanied for some distance into the free portion of the bract by a few scale bundles.

Taxus presents some features which perhaps ought to be mentioned. The single ovule is produced terminally on a secondary dwarf branch clothed with a few pairs of decussate bracts. The primary dwarf branch may occasionally become a long branch by the resumption of growth by its terminal bud. In all of the many ovules examined the ovule is flattened transversely to the uppermost pair of bracts. The four final bundles of the branch of the axis which fuse in pairs before entering the two wings of the ovule fuse in pairs across the next lower pair of bracts, and not across the uppermost pair of bracts, a behavior which is contrary to what should be expected if the fused bundle were destined to supply an axillary structure. The dying out of bundles near the tip of the axis and the consequent failure to supply the uppermost bracts or enter into the formation of the ovuliferous supplies, as the case may be, suggest that a general reduction and loss of parts is taking place. The terminal position of the ovule, the flattening of the ovule transversely to the uppermost bracts, and the fusion in pairs of the final bundles of the axis in the definite way to form the two bundles of the wings of the ovule suggest a structure which might result from a process well under way in *Juniperus communis*, namely the fusion of sporophylls to form a single structure. This in *Taxus* would imply the reduction of the ovules to one, the complete fusion of two sporophylls to the integument of the ovule, and finally the reduction of the vascular supply of each sporophyll to the single weak bundle present in the wing of the ovule. In view of the modifications that are apparently taking place in other conifers such a course of events may be possible, but further investigation is necessary.

The ovule of *Phyllocladus glauca* presents a slightly simpler situation than that of *Taxus*. The two bundles beginning from the sides of the gap and ending at the base of the ovule are probably the only vestige of the scale. The aril may be no more than an outgrowth such as appears in connection with the ripening of ovules in the podocarps. The outer integument of *Torreya* may be a more complex organ.

If any probable conclusions can be made concerning the ovulate structure of the Taxineae, they must in any event be preceded by a more thorough investigation of the different forms.

In spite of striking modifications, the origin of the megasporophyll is homologous throughout the conifers. As to the identity of the organ in question there seems nothing new to be added. The gradation from foliage leaves to bracts of sporophylls is so definite in many forms, as in *Larix* and *Pseudotsuga*, that the homology of one with the other need not be questioned. The scale is the organ in doubt. Its axillary position and the origin of the vascular supply when separate from that of the bract justifies the theory that it is some modification of a fertile branch. Through shortening of the axis and rather delayed development of the shoot in general, together with a relatively earlier development of the ovules, the semicircle of bundles at the base of an ordinary shoot failed to form the cylinder, but instead flattened out into an arc and in some cases even into a straight line.

Summary

1. In the evolution of the ovulate strobilus in members of the Coniferales, two general tendencies are apparent: (1) the reduction in number of sporophylls in the strobilus; (2) the modification of a compound sporophyll into an apparently simple sporophyll; the latter appears in diverse disguises, but in general implies loss of one of the sporophyll members or welding of the two.

2. Strobilus reduction has reached its highest expression in members of the Cupressineae, Taxineae, and Podocarpineae; one type of strobilus reduction is represented by the general sterilization and reduction of parts in the lower sporophylls of *Pinus*.

3. Simplification of a compound sporophyll has been attained to fullest extent in *Arthrotaxis selaginoides*, *Agathis*, and *Saxegothaea*, and possibly others; an extensive reduction of bract occurs in *Cedrus Libani* and the lower sporophylls of *Pinus maritima;* the scale in *Phyllocladus* is probably reduced so as to be represented only by a distinct ovular supply; the welding of the two organs is complete in *Juniperus communis* and *Chamaecyparis Lawsoniana.*

4. Fusion of bract and scale vascular supplies does not directly parallel fusion of bract and scale.

5. Separate origin of bract and scale vascular supplies occurs most generally in the Podocarpineae and Abietineae; fusion of bract and scale supplies has reached its highest expression in the Araucarineae; both types of bundle origin are represented in the same strobilus in *Cryptomeria japonica*, *Cupressus Benthamii*, and the lower sporophylls of *Pinus*.

6. The bract bundle in plants with uninerved vegetative leaves divides only slightly if at all; the extent of the scale bundle system is directly related to the size of the organ supplied.

7. The scale bundles in the Abietineae and *Chamaecyparis Lawsoniana* form in the expanded portion of the organ a straight row or arc; in members of the Taxodineae and Cupressineae scale bundles swing around so as to lie at each side of the bract bundle.

8. In *Cryptomeria japonica* and *Cupressus Benthamii* and perhaps *Cunninghamia Davidiana* scale bundles accompany the bract bundle into the free portion of the bract.

9. A branching bundle in the vegetative leaf in *Araucaria* and *Agathis* probably implies a branching bundle in the bract of the sporophyll; the vascular system in the megasporophyll is probably a complex of bract and scale bundles.

10. In species of *Podocarpus* the scale bundles continue in the portion of the scale folded toward the dorsal side, forming the epimatium of the ovule.

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STUDIES OF DIOSPYROS KAKI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 209

(WITH PLATES XII AND XIII AND ELEVEN FIGURES)

KONO YASUI

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STUDIES OF DIOSPYROS KAKI. I

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 209

KONO YASUI

(WITH PLATES XII AND XIII AND ELEVEN FIGURES)

Although the Ebenaceae are recognized as a primitive family of the Sympetalae and possess several remarkable features, the only morphological investigation of them has been that of Miss HAGUE[1] on *Diospyros virginiana*. My purpose in undertaking the investigation of *D. Kaki* was to answer the following questions: (1) Are the Ebenaceae really primitive among the Sympetalae? (2) Are there cytological differences among the so-called garden varieties of *D. Kaki?* (3) How are the garden varieties related to one another? (4) How have they been obtained?

Material and method

D. Kaki is in very common cultivation in Japan and is represented by many so-called garden varieties. The fruits of these varieties differ in shape, size, and flavor, and also in their seeds. Each variety has also its characteristic flowers, leaves, etc., to which horticulturists paid little attention in classification, as compared with the differences in fruits and seeds. For example, the horticultural classification recognizes two principal divisions, dependent upon flavor of the fruit, as follows: (1) astringent kaki (shibu-kaki), and (2) sweet kaki (ama-gaki).

Each of these two divisions is divided into four or five groups, based upon the shape of the fruit, as follows. The astringent group contains (*a*) long, (*b*) round, (*c*) square, and (*d*) flat types; while the sweet group contains (*a*) gosho, (*b*) long, (*c*) round, (*d*) square, and (*e*) flat types.

In her report on *D. virginiana*, Miss HAGUE makes the following statement: "So far as the trees from which material was collected

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[1] HAGUE, S. M., A morphological study of *Diospyros virginiana*. BOT. GAZ. 52:34–44. *pls. 1–3*. 1911.

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were observed, they were dioecious, and bore only perfect flowers. One possible exception has been found recently." In *D. Kaki*, on the other hand, there are many such exceptions, as follows.

1. In some varieties only the pistillate flowers occur in good condition, while all the staminate and some of the pistillate flowers are imperfectly developed.

2. In other varieties (gosho, egosho, yamagaki, jenjimaru) both kinds of flowers are always well developed.

3. A tree in Mr. YABE'S garden in Tokyo (no. 1 of my material) has the same type of fruit as in some of the varieties in group 1, but has pistillate, staminate, and perfect flowers, and produces also two types of fruit from the two kinds of flowers.

4. No tree has been discovered by the writer on which only staminate flowers occur.

5. Some varieties have pistillate flowers only, when the trees are young, but as they become older or weaker (on account of poor nutrition or transplanting) they begin to produce staminate flowers also (hyakume-gaki).

6. Some trees show an alternation of fruiting and non-fruiting years, which means that during one year they produce many pistillate flowers and many fruits, while during another year they produce a few pistillate flowers and abundant staminate flowers. Under the latter conditions they bear very little fruit, or no fruit at all.

It may be added, in reference to the fourth condition referred to, that in Japan the staminate trees are being continually destroyed unconsciously, because the better varieties are produced always by grafting, and therefore many seedlings of staminate plants are destroyed, and pistillate plants take their place. The foregoing data gave me the suggestion that *D. Kaki* is naturally a monoecious plant (fig. 1), and that it is in process of losing this character, producing staminate flowers under cultivation.

I have selected five different varieties from one hundred or more for this study, as follows.

NO. 1.—I am not certain of the name of this variety, but it resembles "yemon," which is an astringent, flat type. The difference is that yemon has no staminate flowers at the Okitsu station, but this form has three kinds of flowers upon the same individual.

No. 2.—Tenryubo, which is a sweet, round type. It has no staminate flowers at the Okitsu station. The fruit is rather small and has many seeds.

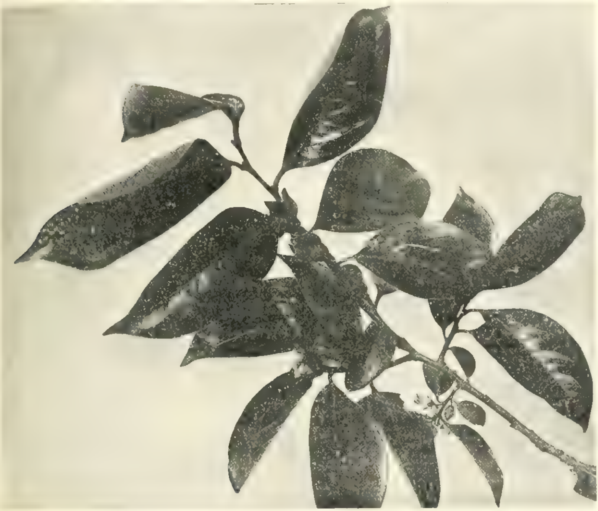


FIG. 1.—Branch of Tsuru-no-ko (one of the varieties of *D. Kaki*), showing the vigorous upper branchlets with pistillate flowers and the lower small branchlets with staminate flowers; $\times\frac{1}{2}$.

No. 3.—Jenjimaru is a sweet round type; but in this case there are always staminate flowers. The fruit is small and with many seeds, as in no. 2.

No. 4.—Tanenashi (meaning seedless) is an astringent square type. It has no staminate flowers and is generally seedless.

No. 5. Fuyū is a sweet, gosho type. Usually it has no staminate flowers, but occasionally a very few may be produced. Occasionally the anthers of the perfect flowers contain a number of pollen grains, which germinate well on the stigma and in nutrient fluids.

I have also examined many other specimens, but the main results of the investigation were obtained from these five. The material of no. 1 was collected in Tokyo, but the others were obtained from the experiment station at Okitsu, in Central Japan. The material was fixed with Flemming's weaker solution, and chromo-acetic acid mixture was also used, giving better results than the former medium. The sections were stained with Flemming's triple stain and Haidenhain's iron-hematoxylin.

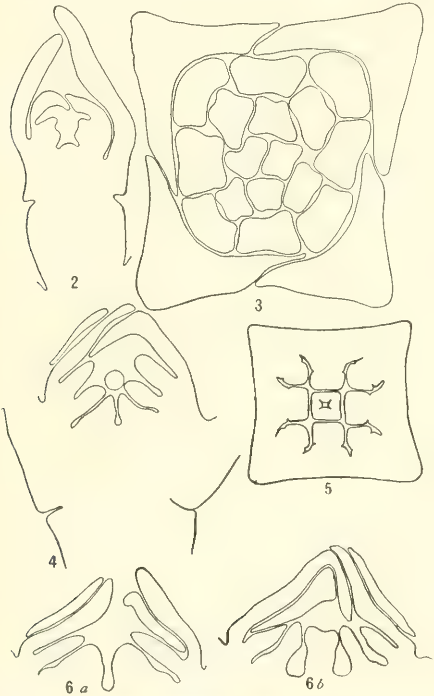
Development of staminate flower

The cluster of staminate flowers appears on young shoots in early spring. It consists usually of three flowers (fig. 1), but sometimes includes 7 or more. The very young shoot bends downward, and 2-4 of the lower leaves have nectaries on the under surface, near the base. The succession of members is strictly centripetal. The calyx is valvate, but the four petals are spirally arranged (fig. 3). The 16 stamens occur in four cycles (fig. 3), and appear in pairs from the earliest stage (fig. 2). The mature stamen has a very short filament, and the connective extends above the anther. The pistils complete their development very early. A few flowers were found which were 5-merous instead of 4-merous.

Development of perfect flower

The perfect flower always occurs at the top of the cluster. It has the same arrangement of members as the staminate flower when young (fig. 4), and the pistils of the perfect flower are smaller than those of the pistillate flowers. Occasionally they have less than 8 ovules, which is the ordinary number in the pistillate flowers, and sometimes they have no ovules. Abnormalities occur, not only in the number of ovules, but sometimes in the irregular forms of the pistils themselves. The fruit of the perfect flower is always smaller than that of the pistillate flower, and usually it has no seeds.

The foregoing facts suggested that the perfect flower is an abnormal one, produced by favorable conditions of nutrition; and that therefore it does not indicate a general tendency.



FIGS. 2–6.—*D. Kaki:* fig. 2, longitudinal section of a young staminate flower of no. 1; fig. 3, transverse section of a young staminate flower of no. 1, with calyx removed; fig. 4, longitudinal section of a perfect flower of no. 1, with calyx removed; fig. 5, transverse section of the lower part of a pistillate flower of no. 4, showing the 8 anthers and a pistil; fig. 6, flower of no. 4: *a*, radial section of a young pistillate flower through the two large opposite protrusions; *b*, longitudinal section through the two initial papillae on the large protrusion.

Microspore formation

There is no marked difference between the primary sporogenous cells and the primary wall cells before the latter begin to divide. Two periclinal divisions give rise to three outer wall layers and a tapetal layer. Two or three successive divisions separate the primary sporogenous cells from the mother cells. At the same time the uninucleate tapetal cells become very distinct between the sporogenous tissue and the wall layers, the latter of which become more and more flattened.

During the comparatively long resting period, the spore mother cell increases in size, its protoplasm becomes denser (but not so dense as that of the tapetal cells), and the nucleus becomes large (pl. fig. 1). The nucleus contains rather a small amount of chromatin granules associated with the fine linin network, and also a remarkably large nucleolus and several small ones (pl. figs. 2, 3).

In some cases I observed the larger chromatin granules appearing in pairs, but this did not seem to be the usual situation. The reticulum is denser at the outer part of the nuclear cavity than within. During presynapsis certain connections between the chromatin granules disappear, so that the reticulum gradually becomes simpler in structure. At the maximum of synapsis the chromatin substance appears like a mass of granules (pl. figs. 4 and 5).

There is no morphological connection between the chromatin reticulum and the nucleolus, although they seem to occur in very intimate relationship. A great many nucleolus-like bodies were observed close to the chromatin thread, near the large nucleus, but in other cases there was no such relationship (pl. fig. 4). In the presynapsis stage the double nature of the chromatin thread is not clear, but it becomes gradually evident with the loosening of the thread (pl. figs. 6, 7).

Before 1895, when Moore called attention to the synapsis stage as an important period in the history of the nucleus, this stage was ignored. Since that time it has attracted chief attention, and is regarded in general as an important event in the history of the nucleus. Some investigators, however, do not regard it in this light. For example, LAWSON[2] makes the following statement.

> During their development, however, there is a great accumulation of sap within the nuclear cavity, which causes a great osmotic pressure. The pressure, acting from within, causes the nuclear cavity to expand. This expansion continues until the nuclear cavity grows to twice or even three times the original size. As the growth proceeds the membrane is gradually withdrawn from the chromatin mass within. The result of this withdrawal of the nuclear membrane is the formation of a large clear area of nuclear sap containing the mass of chromatin which has been left at one side. No evidence whatever was found to show that any contraction of the chromatin had taken place.

In my material of *D. Kaki* I could not recognize such a remarkable enlargement after the chromatin reticulum began to separate from the nuclear membrane. The great enlargement of the nucleus occurs in the resting stage, so that there is no conspicuous difference in the size of the nucleus at the synapsis stage and at the end of the resting stage, as LAWSON indicates.

The synapsis stage is a normal occurrence, and during it there is some rearrangement, but not fusion of chromatin bodies, which results in the spireme stage. At the beginning of the spireme stage the thread is distributed in the outer cavity of the nucleus, where thickening and shortening occur, resulting in the diakinesis stage (pl. figs. 6, 7, 8). There is no difference in the size of the pairing chromosomes, which are usually parallel, but sometimes X or V-shaped. At the same time there appear in the cytoplasm numerous fibers which penetrate the nuclear cavity and become attached to the chromosomes by one end, in connection with which the nuclear membrane begins to disappear. By the elongation of the fibers the chromosomes, which have been distributed on the surface of the nucleus, are drawn into the center of the nuclear cavity, and there are arranged in an equatorial plate by the gathering of the free ends of the fibers at the poles (pl. figs. 9, 10, 11). The nucleolus disappears entirely during metaphase.

The number of chromosomes is difficult to count by reason of their small size. Usually there are 28 in pairs, but sometimes

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[2] LAWSON, A. A., The phase of the nucleus known as synapsis. Trans. Roy. Soc. Edinburgh 47:591–604. *pls. 2.* 1911.

27 in no. 1 (pl. fig. 11). In *D. virginiana* Miss HAGUE counted 30 or more.

The longitudinal division of the chromosomes in preparation for the homotypic division occurs in metaphase, and at telophase the chromosomes become arranged in the spireme and spread over the outer cavity of the daughter nucleus. The number of chromatin granules is not the same as the *x* number of chromosomes; and there appear two or three nucleoli in the daughter nucleus (pl. fig. 12, *a*, *b*). The homotypic division of the microspore mother cell occurs in the usual way. The two axes of the spindle fibers of this division are not in any definite relation to one another (pl. figs. 13, 14). In wall formation the microspores are in tetrahedral arrangement (pl. figs. 15, 16), and the mother cell wall, which becomes mucilaginous and separates the four young microspores (pl. fig. 17), disappears gradually with the formation of the thick wall of the pollen grain.

At the same time the uninucleate tapetal cells enlarge and become multinucleate. These divisions of the tapetal nuclei are mitotic at first, but later they become amitotic, after which they begin to disorganize when the homotypic division of the mother cell has been completed (pl. figs. 20, 21, 22).

The mature pollen grain is spherical, with remarkable canals upon the surface of the wall (pl. fig. 18). The cytoplasm occurs as a thin layer surrounding a large central vacuole. In general only a single nucleus was observed in the pollen grain, pl. fig. 19 representing the only specimen in which two nuclei were observed.

The foregoing account of microspore formation was obtained from material of no. 1. I have investigated no. 3 also, but could not determine the number of chromosomes. The pollen grain is a little larger than in the case of no. 1. The anther of no. 3 contained many withered pollen grains.

Development of pistillate flower

The pistillate inflorescence usually appears upon an upper branch, consisting of one flower and two bracts that suggest the position of flowers that have disappeared. The bracts usually fall after blooming, but in some varieties they persist even after the ripening of the fruit.

The staminate flower begins to develop as early as the pistillate, but the pistils develop more rapidly than the stamens, resulting in the sterilization of stamens and in some cases in their disappearance.

The mature pistillate flower generally has two sets of stamens (nos. 1 and 2), but in some cases has more (nos. 1, 2, and some of 4) or less (no. 1 and some of no. 2).

I did not discover the following condition described by Miss HAGUE: "In the pistillate flower it is a common occurrence to find the number increased by the branching of one or more of the stamens."

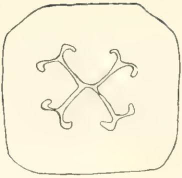
In the development of the pistil there appear first four large protrusions, and then four smaller ones between them (figs. 5 and 6, *a*, *b*). The larger ones give rise to two small papillae, which are the initials of ovules (figs. 6, *d*, and 7). The papillae grow at first toward the wall of the ovary, then curve downward toward the center, and finally upward (figs. 8 and 9). Simultaneously the small protrusions develop toward the center of the ovary, resulting, along with the development of larger protrusions, in 8 loculi for the 8 ovules.

The ovule has two integuments (fig. 9), which extend beyond the nucellus like a beak at the time the embryo sac is fully developed (fig. 10).

Megaspore formation

In all the varieties examined the archesporial cell is solitary and hypodermal. There is no division resulting in a parietal cell, so that the archesporial cell is the megaspore mother cell. The heterotypic division of this nucleus occurs in the same way as in the case of the nucleus of the microspore mother cell. After nuclear division, the cytoplasm organizes into two parts, the inner one being the larger. Sometimes a wall between these two parts does not appear before another division of nuclei occurs.

Wall formation in connection with the megaspores is variable in direction. Sometimes the two walls are perpendicular to one



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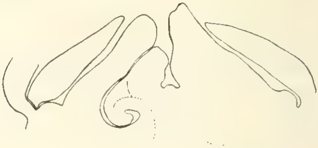

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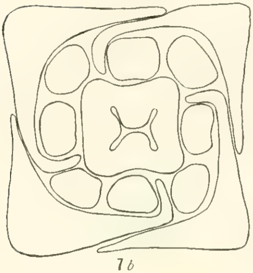

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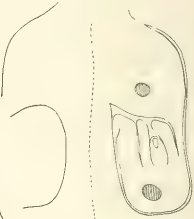

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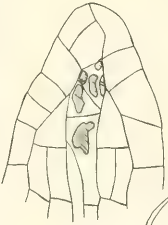

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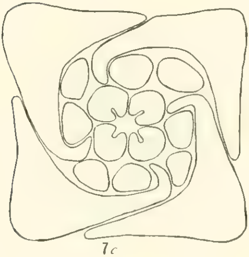

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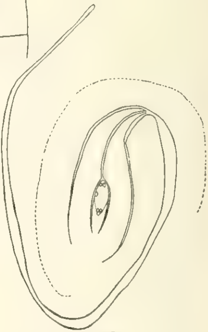

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FIGS. 7–11.—*D. Kaki:* fig. 7, cross-section of a pistillate flower of no. 4, at a little older stage than in figs. 5 and 6: *a*, lower part, showing 4 large protrusions which have papillae, and small protrusions among them; *b*, portion of the upper part, showing 4 petals, 8 anthers, and upper part of ovary; *c*, a still higher part, showing 4 petals, 8 anthers, and 4 young stigmas; fig. 8, longitudinal section of a young pistil of no. 4; fig. 9, longitudinal section of the central part of an ovary of no. 4, showing the longitudinal section of an ovule; fig. 10, longitudinal section of an ovule of no. 4; fig. 11, longitudinal section of the upper part of a nucellus of no. 4, showing the disorganizing embryo sac mother cell.

another, but at other times more or less parallel, resulting in different arrangements of megaspores (pl. figs. 23–26). The micropylar megaspores degenerate in connection with the maturation of the large innermost one in forming the embryo sac (pl. fig. 27). In my material I observed all of the megaspores disorganizing (fig. 11), and it is evident that this disorganization of the megaspore which ordinarily gives rise to the embryo sac is one reason for seedless fruit. In such a country as Japan, where many different kinds of persimmons are cultivated together, the difficulty of pollination is out of the question.

Summary

1. *Diospyros Kaki* is not a dioecious plant but a monoecious one whose staminate flowers are disappearing under cultivation.

2. The monoecious habit might have been derived from a condition of perfect flowers; therefore this habit is not a primitive character in this species.

3. Perfect flowers do not indicate the primitive character of the variety in which they occur; they appear among other varieties only through restoration of lost parts.

4. The primitive character of Ebenaceae among Sympetalae is indicated by the spiral arrangement of petals; the stamen situation, although the number of stamens is not definite; and the two integuments.

5. Megaspore formation is also of a primitive character, and suggests, along with other characters of the family, that it may have some relation to the Myrtiflorae.

6. There is no parietal tissue in the microsporangium, which indicates that Ebenaceae come from some higher family of Archichlamydeae, because in the lower families parietal tissue usually occurs in the megasporangium.

7. Embryo sac formation occurs in the usual way, and in general furnishes no evidence for the evolution of dicotyledons. It is true in general, of course, that the gametophytes of angiosperms are of less value for evidence concerning evolution than the sporophytes.

8. The $2x$ number of chromosomes is 56 or 54.

I desire to express my thanks to Professor JOHN M. COULTER, under whose direction the investigation was undertaken; to Professor C. J. CHAMBERLAIN, who gave much advice; and to Mr. Y. KUMAGAI and Professor Y. YABE, who assisted in securing material.

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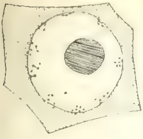
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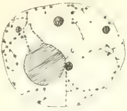
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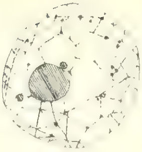
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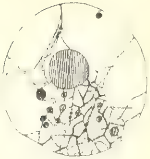
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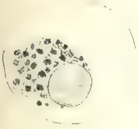
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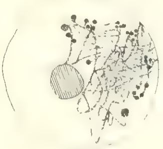
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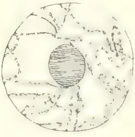
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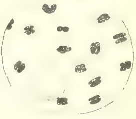
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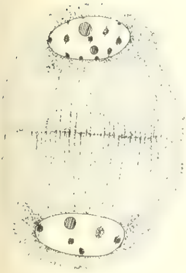
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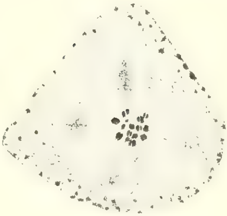
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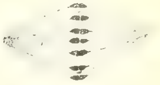
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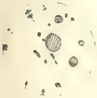
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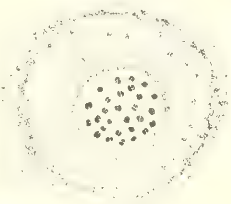
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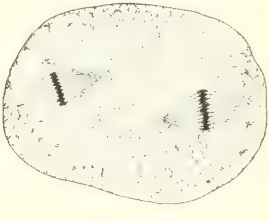
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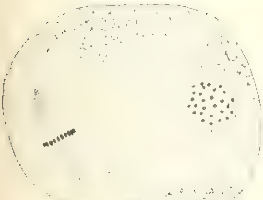
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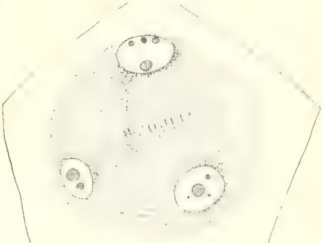
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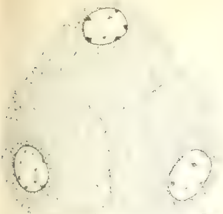
YASUI on DIOSPYROS



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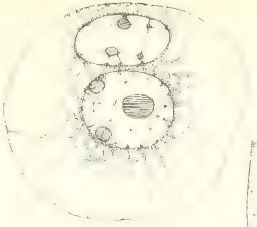

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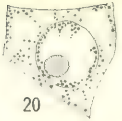

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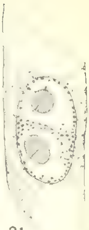

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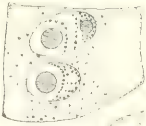

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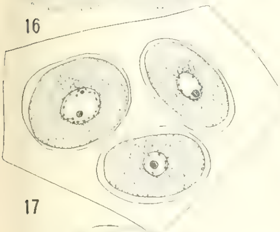

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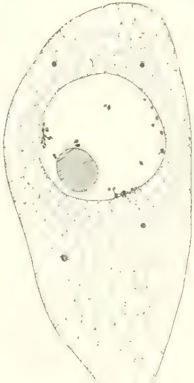

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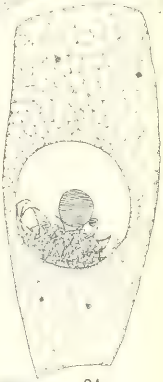

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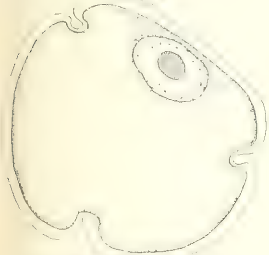

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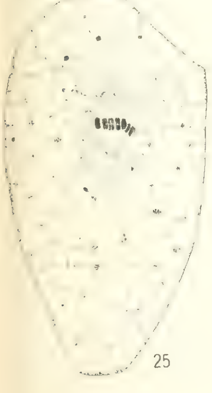

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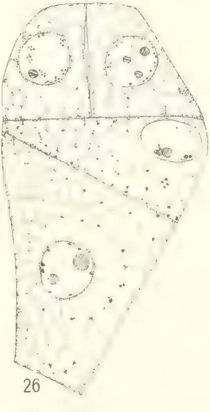

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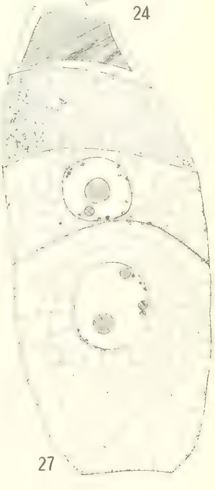

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FERTILIZATION IN ABIES BALSAMEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 210

(WITH PLATES XVI-XX AND ONE FIGURE)

FROM
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A. H. HUTCHINSON

(WITH PLATES XVI–XX AND ONE FIGURE)

A general account of fertilization and the related phenomena in this species has been given by MIYAKI (**19**). A number of supernumerary nuclei were noted in the micropylar end of the egg at the time of fertilization. These were generally regarded as derived from the male gametophyte, while in some cases another nucleus, that of the ventral canal cell, was added to the number. Repetition has been avoided, inasmuch as the present account is restricted to special problems, while the general account has received little attention.

The problems suggested by the regular occurrence of four nuclei in the egg cytoplasm, near the micropylar end, at the time of the 4-nucleate proembryo, and by the unusual grouping of chromosomes during the division which follows the conjugation of the egg and sperm nuclei, have led to this investigation. Special attention, therefore, will be given to the fertilization of the nucleus of the ventral canal cell and to the cytological features connected with the fertilization of the egg.

The material was collected in Ontario, Canada. I am greatly indebted to Professor W. R. SMITH for collections made at Lake Joseph. On June 25 and 26 I obtained ovules showing fertilization at N. lat. 44°, W. long. 79°12′, and on July 2 and 3 ovules showing similar stages at N. lat. 45°30′, W. long. 78°32′. The altitude in the first case was 900 feet; in the second 1800 feet.

In a former paper on the male gametophyte of *Abies* (**10**), the excessive number of prothallial cells has been recorded. One of these polar cells, during mitosis, is shown in fig. 1. Fig. 5, which was drawn from a pollen grain lodged in the micropyle, shows the division of the body cell nucleus, to form two male nuclei, taking place before the tube breaks through the spore coat. At this time the cytoplasm surrounding the tube nucleus is extremely vacuolate, that of the body cell very dense. The tube nucleus begins to disintegrate before the pollen tube emerges from the exine; usually during the pollen tube stage it appears only as an irregular aggregation; seldom, if ever, does it enter the egg.

A number of gametophytes have been found similar to that shown in fig. 2. The first division has cut off a polar ("prothallial") cell which later has disintegrated; the second division has given rise to two equivalent cells; which of the two might have been regarded as prothallial, under other circumstances, is impossible to determine. Evidently there are two antheridia. The conclusions based on a study of *Picea* (**9**), namely, that under favorable conditions any of the cells resulting from the three primary divisions of the male gametophyte may be antheridial, are supported by the facts as found in *Abies*.

The period of time between pollination and fertilization is from four to five weeks. During the greater part of this time the pollen grains lie dormant on the nucellus, or lodged in the micropyle. The course of the pollen tube is direct, and the motion rapid. Although the rate is difficult to determine, it is believed that the passage time of the pollen tube does not exceed two days, and probably may be measured in hours.

Fertilization of the ventral canal cell

The division of the central cell to form the egg and the ventral canal cell is similar to that in *Pinus* (figs. 7, 8, 9; compare, 3, 5, 6, 19); the nuclei formed are similar in size and form. The egg nucleus at once begins to move toward the center of the egg cytoplasm (fig. 7). What the attractive force may be is unknown, but the nucleus of the ventral canal cell responds to the same force and moves in the same direction. This nucleus breaks through the cell wall and enters the cytoplasm of the egg (figs. 11, 12); here it increases in size until it reaches a length, in some cases of 80 μ, which is approximately one-half of the greatest length attained by the egg nucleus. The structure of the ventral nucleus is very similar to that of the egg, which will be described later. The latter is surrounded by a dense granular layer which is not present in the case of the former.

The fertilization of the nucleus of the ventral canal cell has been seen in several instances. One of the male nuclei fuses with the ventral nucleus (figs. 15, 17, 21); the stalk nucleus also may be in close proximity (figs. 17, 18). Sometimes two tubes enter the archegonium, in which case male nuclei from different gametophytes may fuse with the ventral nucleus and egg nucleus respectively (fig. 21). The chromatin of the fusion nucleus condenses near its center (fig. 18), and the first division takes place. Two successive divisions (figs. 16, 23, 24, 26) result in four nuclei, which as might be expected, are generally arranged in pairs (figs. 22, 25). The nuclei of this ventral proembryo range in diameter from 40 to 50 μ; those of the proembryo proper from 60 to 65 μ; otherwise the similarity is very marked (figs. 32, 53, 54).

Nuclear changes

The changes in size of the nuclei located in the egg cytoplasm are the most readily measured of all the modifications; moreover, the increase or decrease in volume will serve to indicate the extent of the qualitative changes which occur. Immediately after the division of the central cell the egg nucleus measures about 30 μ in diameter (fig. 7). At the time of fusion its length approximates 100 μ (fig. 19); during the approximation of the chromatin groups 160 μ is the maximum measurement (fig. 27), and during anaphase of the first division the diameter is again reduced to 50 or 60 μ (fig. 46). The changes in size of the daughter nuclei are less marked; the diameter varies from 20 μ at telophase (fig. 52) to 65 μ in the resting stage It is not surprising that the egg nucleus should vary greatly in structure while increasing to 60 times its original volume, and again decreasing to one-tenth its attained volume.

During the early stages of the first division, and even before the chromatin groups have united, four differentiations of the "nuclear" material are evident. The chromatin group or groups occupy less than one-tenth of the space within the nuclear membrane (figs. 27, 28, 42). The spindle fibers are intranuclear in origin (fig. 28). Large, vacuolate, irregular, deeply staining masses are distributed throughout the whole area. The greater part of the nuclear cavity is pervaded by slender filaments, which include small granules. An attempt has been made to trace these structures from their origin to their fate, in order that something regarding their nature and function might be determined. This complexity of structure is in contrast with the prophase of an ordinary mitosis, where only chromosomes, at most chromosomes and the nucleolus, are inclosed by the nuclear membrane.

The dark vacuolate masses are most conspicuous just after fusion (fig. 27). In the early stages small refractive globules are scattered throughout the granular egg nucleus; later, these become inclosed in a gelatinous network (fig. 19), and next appear as previously described. During the first mitosis they accumulate into several globular, vacuolate bodies (fig. 34). The latter decrease in volume or become distributed throughout the nucleus during metaphase (fig. 46). At anaphase the irregular masses once more become conspicuous (fig. 51). When the daughter nuclei are formed, these bodies are not included, and soon disappear. The fact that they are extruded would seem to indicate that they are not fundamental nuclear material. The fact that they are stored up during the growth of the nucleus and decrease in amount during mitosis would suggest that they are simply stored food bodies.

Two successive groups of intranuclear fibers become differentiated during the processes of fusion, and the first division after conjugation. The first is concerned with the approximation of chromosomes to be described later; the second with the first mitosis. While the two chromatin groups, from the egg and from the male nucleus, are still distinct, fibers which penetrate and surround these groups are organized (fig. 28). A union of the two groups of fibers results in the formation of a single spindle made up of large complex strands (figs. 29–33). The spindle drawn was 60 μ in length. After the approximation of the chromosomes into pairs the spindle broadens; the fibers become less conspicuous, and finally disappear, leaving a group of irregularly arranged chromosomes within the now much reduced nuclear space (figs. 43–45). Meanwhile the chromosomes migrate to the center of the nucleus, and soon the second set of fibers is formed (fig. 46). At first these are restricted to the region in which the chromosomes are situated; as the chromosomes move to the poles, the whole nucleus becomes pervaded by fibers radiating from irregular centers (fig. 51). In the egg of *Abies* there is an excessive development of intranuclear fibers. It seems reasonable to suppose that they originate from cytoplasmic material which has entered the nucleus during its growth period, and which may be differentiated into the fibrous form under physiological conditions not yet determined.

The slender filaments pervaded by small granules are scattered throughout the greater part of the nucleus. They are most definitely organized at the time when the spindle fibers are most conspicuous (figs 27, 33, 42). When the fibers disappear, the filaments become disorganized, resulting in a granular mass (figs. 43, 46); when the spindle fibers appear the second time, the whole nucleus becomes more or less fibrous (fig. 51); when the daughter nuclei are formed, these bodies are not included, but form a matrix for the nuclei (figs. 49, 50). We may conclude that these filaments also are cytoplasmic, resembling the spindle fibers in nature, and becoming differentiated under similar conditions.

Pairing of chromosomes in fertilization

The succession of events which occur in connection with fertilization and the first division of the zygote has been traced not only by a consideration of the stages in the approximation and redistribution of chromatin bodies, but also by tracing parallel series of changes in the size of the nucleus, in the modifications of the deeply staining food bodies, and in the formation and dissolution of spindle fibers. A study of the chromatin, involving as it does the union of the male and female elements and distribution in the daughter nuclei, is of primary importance. Emphasis has been given first to a study of the related phenomena, already described, thereby eliminating, in so far as is possible, the possibility of a misinterpretation of the order of events.

The approximation of male and female nuclei has been described for a number of conifers. In general the process as found in *Abies* agrees with that of these descriptions. A few features may be noted. No cytoplasm could be detected adhering to the male nucleus as it approached the egg nucleus. There is a great disparity in the sizes of the pairing nuclei; that of the egg, as shown in fig. 19, is 120 μ by 80 μ, while the male nucleus is 45 μ by 15 μ. The membranes of the nuclei are resorbed at the place of contact, and the contents of the male nucleus pass into the interior of the female nucleus, thereby leaving a protuberance containing very little cytoplasm.

In *Abies* the chromatin could not be detected definitely until the formation of two groups in the micropylar end of the egg nucleus takes place. While the groups are still distinct, the individual chromosomes become separate (fig. 28), each group containing the haploid number of chromosomes. As the two spindles unite, the chromosomes become paired (figs. 21–33); at first the individuals of a pair approximate side by side (figs. 31, 32); soon they twist about one another and jointly loop into the form of a C (figs. 30, 32, 33). The chromosomes are very large, in some cases exceeding 20 μ in length. There is abundant evidence that this is a pairing, not a longitudinal splitting of chromatin elements. First, the number of pairs is haploid. This is the number which would necessarily result from a pairing of the double number of chromosomes already present; a splitting would, of course, result in $2x$ pairs. Also, the twisting of the chromosomes about one another is identical with their behavior in what is generally regarded as a pairing during the prophase of the first reduction division. Moreover, there follows a transverse segmentation. If the diploid number of chromosomes should undergo two divisions, one longitudinal and one transverse, an $8x$ number of chromosomes would necessarily result. The facts cannot be explained by the supposition that there is a longitudinal split; they are readily explained by regarding this paired appearance as a true pairing.

The segmentation.—The bending into C or V-shaped forms is followed by a segmentation of each component of the pairs; in other words, a transverse fission at the angle of the bent chromosomes (figs. 34, 40). The resulting $4x$ daughter segments are approximately 10 μ in length, or one-half the length of the pairs before and during the looping process. At first the segments remain more or less twisted about one another (figs. 34, 37, 41, 42), and for some time retain a paired relation (figs. 43, 45). They may be in the form of X's, or V's, or parallel rods. At the time when the second set of spindle fibers begins to be differentiated, the $4x$ number of chromosomes are indiscriminately intermingled (figs. 46–48). Half the number pass to each pole to form the daughter nuclei.

The chromosome count.—Repeated chromosome counts in the sporophyte and gametophyte confirm one another in fixing the x and $2x$ numbers for *Abies balsamea* as 16 and 32. The individual chromosomes which appear during the prophase of the division in the central cell are shown in figs. 8 and 9. The count is 16. The division of the body cell in anaphase gives 39 segments. A reconstruction of parts separated by the microtome knife results in a count of 32, or 16 passing to each pole. At the time of approximation of the chromosomes in the egg, there are 16 pairs (figs. 29–33). When segmentation takes place, 32 pairs of segments are present (figs. 34–40). In the nucleus represented in figs. 43–45, there are 72 chromosome pieces; figs. 46–48 show 63 almost complete chromosomes, besides a number of ends. Undoubtedly we have in each case the $4x$ number, or 64 chromosomes.

The daughter nuclei.—During telophase the chromatin strands remain remarkably distinct (figs. 49–52). They elongate greatly, and become irregularly looped (figs. 50, 52). It would seem that each is in contact with the periphery somewhere throughout its length. Contraction is followed by an increase in the size of the nuclear space; the latter is accompanied by a vacuolization of the chromatin (fig. 55). The nuclear outline is still lobed, the lobes corresponding to the loops of chromatin. The nuclear membrane forms late. As the nucleus continues to enlarge, the chromatin becomes still more discontinuous, but the outline of the strands may still be readily traced.

It is to be noted that of all the material which was inclosed by the membrane of the egg nucleus, only the chromatin is included in the newly organized daughter nuclei. The large vacuolate darkly staining bodies, the filaments pervaded by granules, and the fibers are all excluded when the membranes inclose the daughter nuclei. We may conclude that these materials, although they may be found within the nuclear membrane, are not essentially nuclear and are not directly concerned in mitosis. They are, at most, cytoplasmic inclusions within the nucleus. The chromatin is the fundamental nuclear substance.

Discussion

The ventral canal cell.—The general tendency among the Coniferales is toward the reduction of the ventral canal cell. In the Abietineae a cell is cut off by a cell wall; in the Taxineae and Cupressineae, as groups, a ventral nucleus is formed, but no cell is organized; while in *Torreya* (**15**) there is no ventral cell, the nucleus of the central cell becoming the egg nucleus. In *Abies* the nucleus of the ventral canal cell functions as an egg. In *Pinus Laricio* (**3**, p. 278), "while the ventral canal cell nucleus usually disappears soon after it is formed, in some cases it persists, and its nucleus becomes as large as that of the oosphere, passing through a similar developmental history. New support is thus given to the theory that the ventral canal cell is the homologue of the egg."

NICHOLS (**21**) describes (fig. 90) "two nuclei resulting from the division of the ventral canal cell nucleus" in *Juniperus*. The ventral canal cell "is fairly persistent in *Tsuga* (**20**). When division is complete, its nucleus is equal in size and similar in structure to the nucleus of the egg, and for some time shows the same stages of development."

The most extreme development recorded is in the case of *Thuja*, described by LAND (**14**). "A number of the writer's preparations of *Thuja* lead him to believe that both the ventral nucleus and the egg, in the same archegonium, may be fertilized. In fig. 17 the proembryo is well advanced, while the ventral nucleus has formed a group of four cells. Another preparation shows eight cells with indications that walls are soon to appear. The probability of such a fertilization is strengthened by finding occasionally in the same ovule embryos growing upward into the nucellus, as well as downward into the endosperm" (p. 224). These facts and those already described for *Abies* remove any doubt that the ventral canal cell is potentially an egg.

The cytoplasmic "mantle" about the egg nucleus is present in most species of Coniferales. NICHOLS (**21**) describes it in *Juniperus* as follows: "The mass of cytoplasm and starch derived from the male cell gradually surrounds the conjugating nuclei so that there is never any possibility of mistaking the fusion nucleus for an unfertilized egg nucleus." And NORÉN (**22**) states: "Nachdem die Kopulation der Kerne erfilgt ist legt sich das Plasma der Spermazelle und deren Stärke wie ein Mantel um die beiden Kerne herum." The description is similar for *Taxodium* (**4**), *Torreya* (**15**), *Sequoia* (**16**), and *Thuja* (**14**). In *Ephedra* (**13**), a dense cytoplasmic mass develops about the egg nucleus and extends downward in the cytoplasm of the egg. In *Abies* a finely granular cytoplasmic layer develops about the egg nucleus during its movement toward the center of the egg (figs. 7, 14). This area extends along the path of the nucleus in the form of a short streamer. The male nucleus penetrates the mantle, but there is no appreciable addition to its mass by cytoplasm accompanying the same (fig. 19). At the telophase of the first division, the excess material from the egg cytoplasm is added to the "mantle" (figs. 49, 50, 55).

The darkly staining bodies described in *Abies* as globular or irregularly shaped and vacuolate, according to conditions, have been variously interpreted. NICHOLS (**21**) writes: "In the egg nucleus frequently the entire chromatin content of the nucleus seems to have resolved itself into nucleoli and pseudo-nucleoli. Yet even after a study of a large number of preparations one is unable to formulate any satisfactory conclusions as to the nature of these structures." Describing the egg nucleus of *Taxodium*, COKER (**4**) says: "In addition to the reticulum and plastin nucleoli there are also present numbers of chromatin nucleoli." And LAWSON for *Libocedrus* (**17a**) states that "It is impossible to distinguish the true chromatin from the nucleoli and other irregularly shaped bodies which stained like chromatin, and seemed to be closely associated with the latter." These bodies seem to resemble very much the karyosomes which SHARP (**24**) has described in the resting nuclei of *Vicia Faba*. "They appear in connection with the chromatic network and resemble the latter in staining quality. They seem to represent an elaboration product of a process actively going on during rest." A study of *Abies* has led to the conclusion that the darkly staining bodies are storage materials derived from the cytoplasm.

A cytological study of fertilization in conifers has been made for a number of species: CHAMBERLAIN, *Pinus Laricio* (**3**); BLACKMAN, *Pinus silvestris* (**2**); Miss FERGUSON, *Pinus* (**6**); MURRILL, *Tsuga canadensis* (**20**); NORÉN, *Juniperus* (**22**); and NICHOLS, *Juniperus* (**21**). "After the male pronucleus is within the oosphere nucleus the chromatin of the two pronuclei appears as two distinct masses in the spirem stage" (**3**). "Es scheint als würde jede der beiden Chromatingruppen zuerst ihre eigen Kernspandel ausbilden, die sich dann zu einzigen vereinigen" (**22**). On the fibers "the long bent and twisted chromosomes appear" in *Tsuga* (**20**). Miss FERGUSON (**6**) states: "When the chromosomes are being oriented at the nuclear plate, the maternal and paternal elements can no longer be distinguished." The number of chromosomes at this stage was found to be $2x$. No count is recorded during the phase of pairing (or splitting, as it has generally been regarded). Miss FERGUSON'S figures confirm what has been described for *Abies*. Her figs. 236 and 237 may be compared with figs. 29–33; her fig. 238 with figs. 34–39; and her fig. 241 with fig. 51. As illustrated by diagram *B*, a number of stages in the process of fertilization have not been described heretofore. It is not surprising that, without these stages and not having the chromosome count throughout, the pairing should be interpreted as a longitudinal split, and that evidence of segmentation should not be found. Because of the complete series found in *Abies* and the extreme size of the chromosomes, it has been possible to discover the facts which center about the pairing, followed by the transverse segmentation.

Stages in fertilization.—A study of the union of egg and sperm in plants and animals makes it evident that there are several phases in the process of fertilization. The primary phase is illustrated by Uredineae. Here the fusion is evidently incomplete during the binucleate stages. "In the young aecidium the nuclei become paired and divide together in very close association. The teleutospores in the young state are binucleate, but when mature become uninucleate by the fusing of the two paired nuclei" (**1**). It is only after many separate but simultaneous divisions of the pairing nuclei that this second phase of the process is accomplished. HARPER (**8**) believes that bivalent chromosomes are formed in

Ascomycetes. "The time and degree of the combination of the sexual chromosomes is a variable matter. If the prochromosomes can remain in one nucleus with the double chromosome number, or in two distinct nuclei through part or all of the sporophyte generation, it is also possible that they may combine in one nucleus into bivalent chromosomes, and maintain their identity in this condition through the sporophyte generation until a true reduction occurs in spore formation." In the well known case of *Cyclops*, studied by Haecker, the parental chromosomes do not mingle, but persist as individuals and maintain their separation into two groups through several cell generations after "fertilization." We may consider the union of egg and sperm as consisting of three stages: (1) the two nuclei enter the same cytoplasmic mass; (2) the two

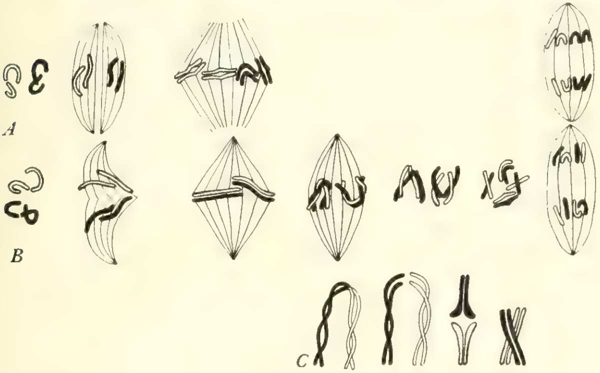

Fig. 1

Diagram *A*.—An interpretation of fertilization in some animals and in *Pinus*, according to the accounts heretofore recorded.

Diagram *B*.—An interpretation of the phenomena already described: the chromatin originating from the egg nucleus is shown in solid black, that from the male nucleus is outlined and barred; the argument upon which such an interpretation is based is included in the description given in the text; the facts of spindle formation are also indicated.

Diagram *C*.—A copy from Grégoire's (7) "schéma de l'interprétation metasyndetique des tétrades-crois," as found in certain lower animals; compare fig. *B*.

groups of chromosomes enter the same nuclear membrane, but retain their former grouping; and (3) the chromosomes pair. The first is illustrated by the paired nuclei in Uredineae; the second by the chromatin groups during the first divisions of the *Cyclops* embryo; the third takes place soon after the fusion of the egg and sperm in *Abies*. It seems altogether probable that the chromosomes do not actually pair until the prophase of the reduction division in such animals as *Cyclops*. There is a striking similarity between the pairing process as described in *Abies* and that concerned with tetrad formation in some animals (compare diagram *A* with diagram *C*). To what extent we are justified in suggesting that the processes are identical and that they are both concerned with the pairing of corresponding chromosomes from the egg and from the sperm, only further research can definitely determine.

Relationships

Abies, as compared with *Pinus*, shows a number of primitive characters:

1. The male gametophyte: (*a*) an excessive polar ("prothallial") tissue; (*b*) the equality of the male nuclei; both may function.

2. The female gametophyte: (*c*) the large number of neck cells; as many as five tiers (MIYAKI **19**); (*d*) the persistence of the ventral canal cell; its nucleus may function as an egg nucleus.

3. The ovulate strobilus and ovules: (*e*) the almost complete separation of scale and bract; (*f*) the development of a rudimentary pollen chamber; (*g*) the comparatively free integuments.

4. The staminate strobilus: (*h*) the staminate strobili are borne on ordinary branches in the axes of ordinary leaves.

5. The arrangement of leaves: (*i*) the spiral arrangement of leaves on ordinary branches (THOMSON **25**; LLOYD **18**).

6. Vascular anatomy: (*j*) the general absence of ray tracheids (THOMPSON **26, 27**), which in *Pinus* are present in the mature wood, but absent in the seedling and strobilus axis.

It may be noted, however, that resin canals are not found in the woody axis of the *Abies* stem except as traumatic responses (JEFFREY **11**). JEFFREY states that the presence of resin ducts is an ancient character which has persisted in *Pinus*. "On account of the reduced foliage of the abietineous conifers, this [that is, resinous secretion] was a very serious drain on the assimilatory apparatus. Gradually the more economical tendency arose of forming resin passages in the case of need only." So regarded, *Pinus* would be more primitive than *Abies*. Whether or not this argument is sufficient to overbalance the numerous ancient characters of *Abies* previously tabulated is a matter of judgment which we do not presume to decide.

Many of the foregoing characters are such that they tend to relate more closely the two ancient groups of Coniferales, the Abietineae and the Araucarineae.

Summary

1. *The male gametophyte.*—The polar ("prothallial") cells may divide mitotically. The body cell divides to form the male nuclei while within the spore coat. Under favorable conditions a "prothallial" cell may develop as an antheridial cell, a biantheridial gametophyte resulting. The male nuclei are equivalent; one fuses with the egg nucleus and frequently the other fuses with the ventral canal cell nucleus.

2. *The ventral canal cell and ventral proembryo.*—The ventral canal cell nucleus breaks through its wall into the egg cytoplasm, enlarges, and fuses with one of the male nuclei. A ventral proembryo is formed by two successive divisions.

3. *The egg nucleus.*—The egg nucleus enlarges to 60 times its original volume. At the time of fertilization (fig. 28) irregular, darkly staining, vacuolate masses, slender filaments pervaded by small granules, spindle fibers, and chromatin are differentiated within the nuclear membrane. The chromatin is the fundamental nuclear substance; the other bodies are accretions gained during the growth of the nucleus and excluded from the nuclei of the proembryo.

4. *Fertilization and the first division.*—After fusion two chromatin groups appear at the base of the egg nucleus; in each $2x$ chromosomes become separate; the two original spindles unite: the chromosomes approximate to form x pairs; they twist the one about the other and become looped; each of the components of a pair

segments medianly, that is, at the apex of the loop; $2x$ pairs of segments result; these separate to form $4x$ chromosomes; a new spindle is formed and $2x$ chromosomes pass to each pole.

5. Fertilization may be regarded as having three phases: (1) sex nuclei enter a common cytoplasm; (2) the two groups of chromosomes enter a common nuclear membrane; (3) the chromosomes approximate in pairs. The first phase may be prolonged, as in Uredineae; the second may be prolonged, as in some animals; or the three phases may follow one another in rapid succession, as in *Abies*. Attention is drawn to the similarity existing between the phenomena connected with pairing in *Abies* and tetrad formation in animals. It is suggested that they may be like processes occurring at different stages of the life history.

I wish to express my thanks for many suggestions and helpful criticisms given by Professor J. M. COULTER and Professor C. J. CHAMBERLAIN, under whose direction the investigation was pursued.

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EXPLANATION OF PLATES XVI–XX

Figs. 1–5.—Pollen grains: male gametophyte.

Fig. 1.—Division of polar cell; ×510.

Fig. 2.—Biantheridial gametophyte; ×510.

FIGS. 3-5.—Division of body cell; ×865.

FIG. 6.—Central cell and two neck cells; ×510.

FIG. 7.—Ventral canal cell and egg nucleus; ×510.

FIGS. 8, 9.—Mitosis of nucleus of central cell; ×865.

FIG. 10.—An archegonium: egg nucleus, ventral canal cell with wall broken and escaped nucleus in the cytoplasm of the egg; ×85.

FIGS. 11, 12.—Nucleus of ventral canal cell escaping.

FIG. 13.—Egg nucleus near a male nucleus; ventral canal cell nucleus, and near by the other male nucleus and stalk nucleus.

FIG. 14.—Detail of egg nucleus of fig. 10.

FIG. 15.—Two archegonia, one showing fertilization of both egg and ventral canal cell nucleus; ×85.

FIG. 16.—Divisions following the two fusions; ×85.

FIG. 17.—Ventral canal cell nucleus: a male nucleus and the stalk nucleus in contact; ×510.

FIG. 18.—Fusion nucleus resulting from union of ventral canal cell nucleus and male nucleus; ×510.

FIG. 19.—Egg nucleus and male nucleus in contact; ×510.

FIG. 20.—Egg and male nucleus in contact (see detail in fig. 19): the ventral canal cell nucleus as described for fig. 18; ×510.

FIG. 21.—An archegonium showing the egg nucleus, ventral canal cell nucleus, four male nuclei, a stalk nucleus, and two pollen tubes; ×510.

FIG. 22.—Postion and relation of ventral proembryo and proembryo proper; ×510.

FIGS. 23-26.—Detail of division following the fertilization of the ventral canal cell nucleus; ×865.

FIG. 27.—Nucleus of the fertilized egg; ×510.

FIG. 28.—Nucleus of the fertilized egg: two groups of chromosomes; ×510.

FIGS. 29-33.—Pairing of chromosomes; ×865.

FIGS. 34-40.—Segmentation of chromosomes; ×865.

FIGS. 41, 42.—Paired segments; ×865.

FIGS. 43-45.—Chromosome segments intermingling, still somewhat paired; ×865.

FIGS. 46-48.—Chromosomes starting to the poles; ×865.

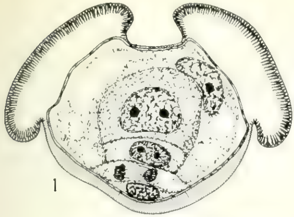
FIG. 49.—Early telophase of first division; ×865.

FIGS. 50, 52, 55.—Late telophase of first division; ×865.

FIG. 51.—Anaphase of first division; ×865.

FIG. 53.—Resting stage of nucleus from ventral proembryo; ×865.

FIG. 54.—Resting stage of nucleus of the proembryo; ×865.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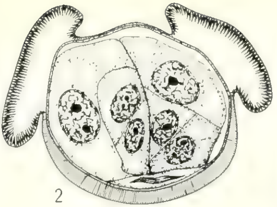
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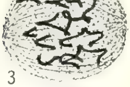
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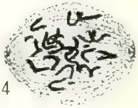
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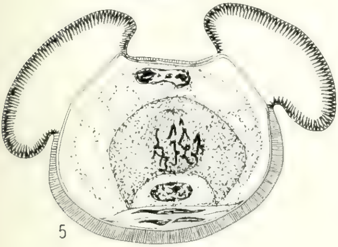
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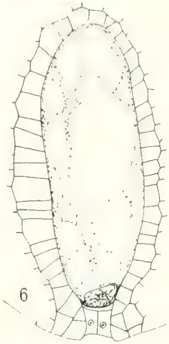
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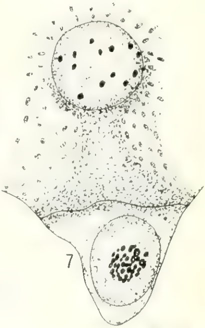
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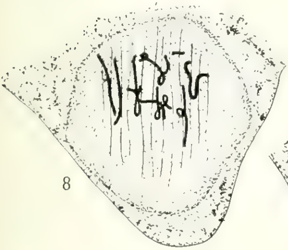
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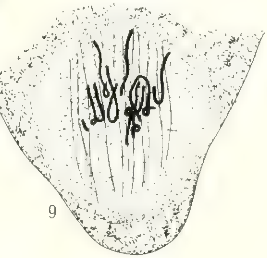
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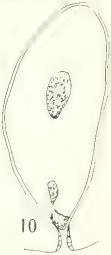
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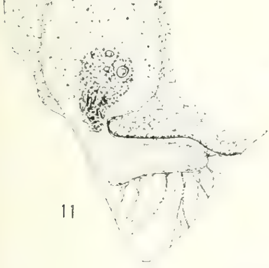
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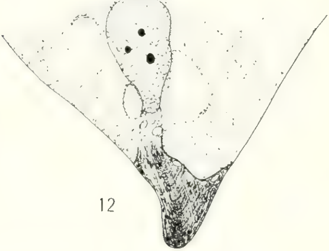
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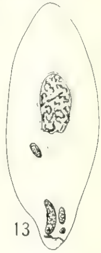
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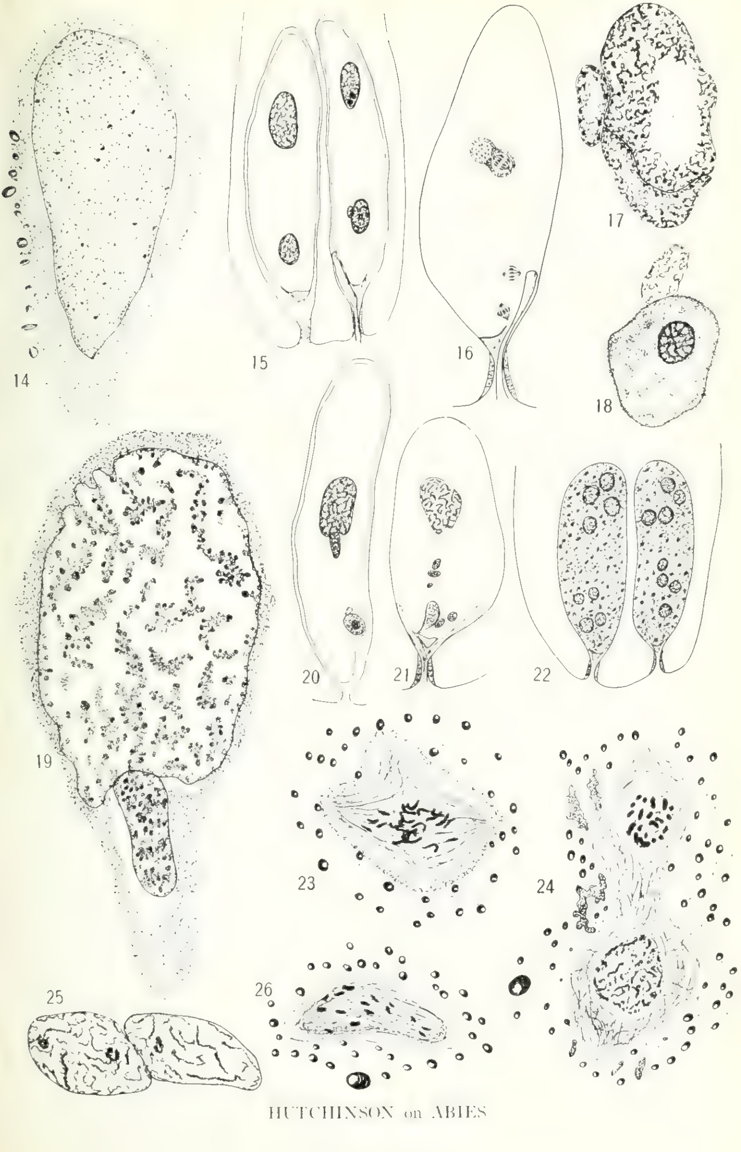

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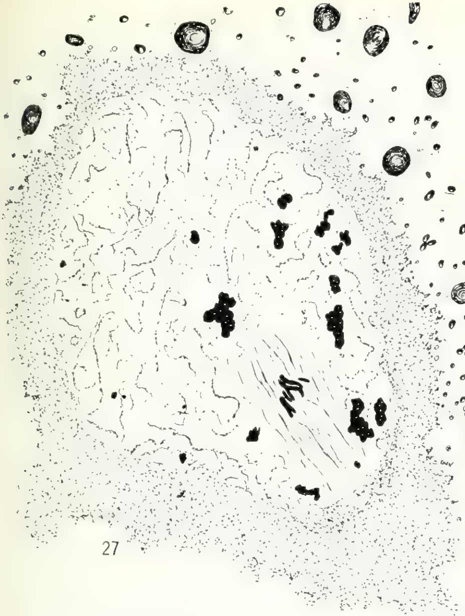
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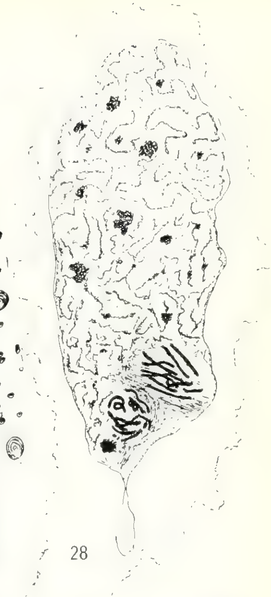
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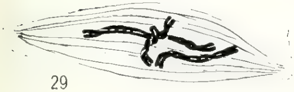
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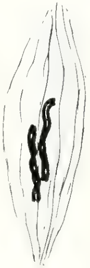
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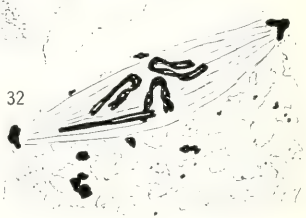
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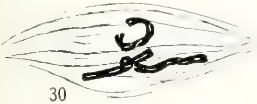
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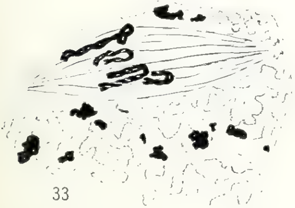
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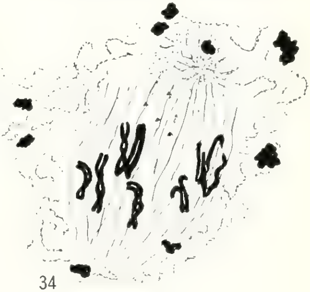
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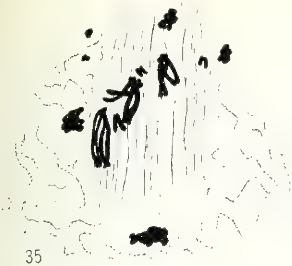
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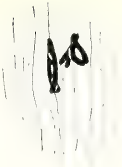
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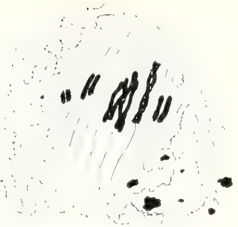
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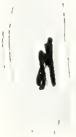
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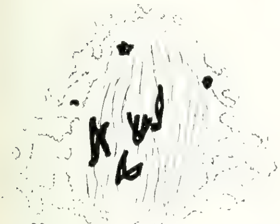
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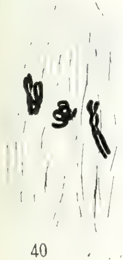
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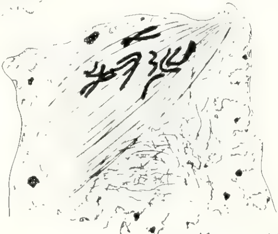
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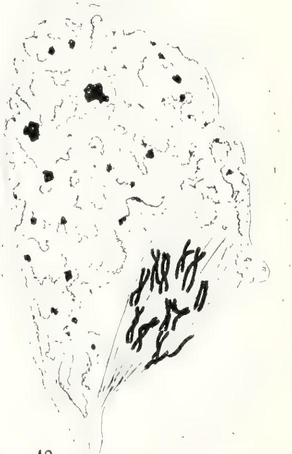
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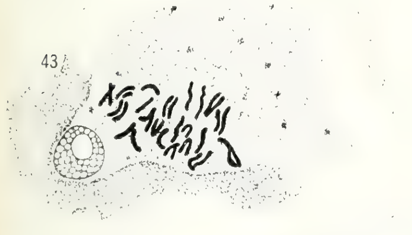
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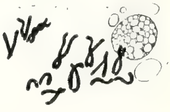
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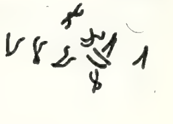
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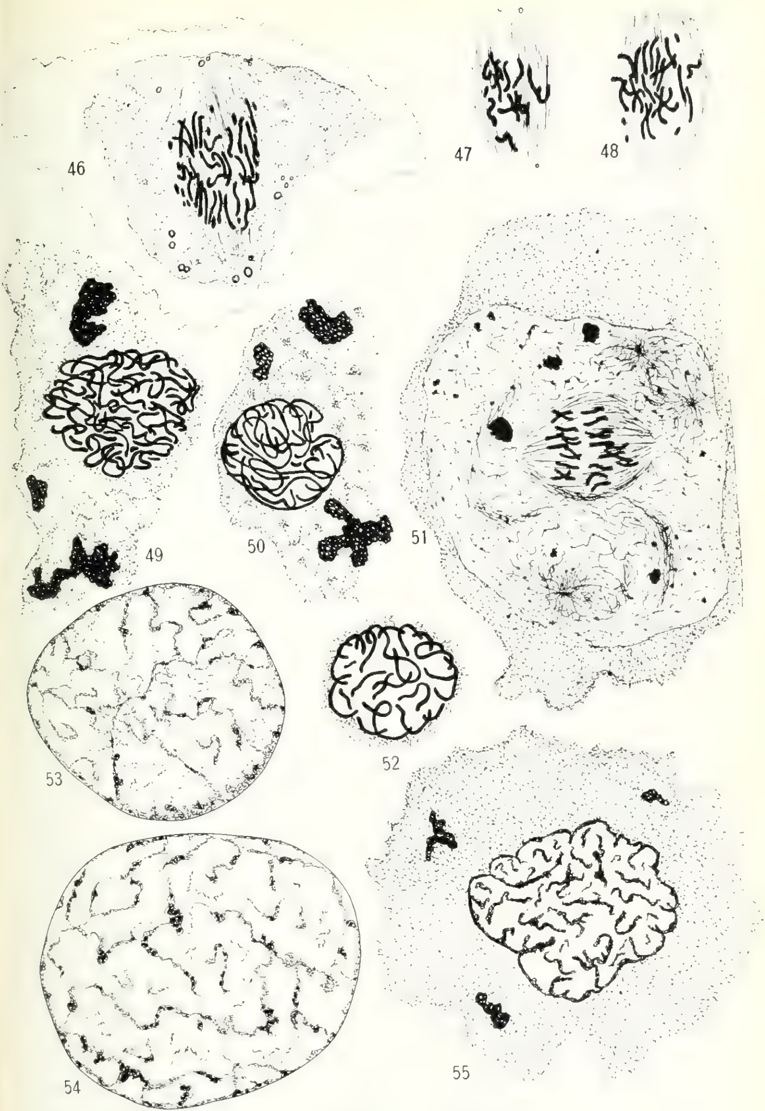

HUTCHINSON on ABIES

A MEXICAN AYTONIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 211

(WITH PLATES I-IV AND FOUR FIGURES)

ANNA M. STARR

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A MEXICAN AYTONIA

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ANNA M. STARR

(WITH PLATES I–IV AND FOUR FIGURES)

To this genus ENGLER and PRANTL refer six names, among which *Aytonia* Forst and *Plagiochasma* L. are most familiar in botanical literature. LEITGEB (14) made a study of *Plagiochasma cordatum*, *P. intermedium*, *P. crenulatum* (Gottsche), *P. appendiculatum*, and *P. Aytonia*. He gives an account of the appearance, the position, and the formation of the antheridial and archegonial receptacles, describing the order of the appearance of the archegonia and the origin of the archegonial cavity and of the involucre. He describes also the protective scales about the receptacles and their origin from a single row of cells, and accounts for the stalk of the receptacle as simply a dorsal outgrowth of the thallus. He concludes with an account of the structure of the sporogonium and its dehiscence.

The material for this investigation was collected in Mexico in 1908 by Dr. W. J. G. LAND and the late Dr. CHARLES R. BARNES. Most of it came from the rocky sides of the deep canyon of the Rio Santiago in the state of Jalisco near Guadalajara, the rest from Carrizal in the state of Vera Cruz. The species cannot be determined definitely. Of the four Mexican forms described by GOTTSCHE, it resembles most closely his *Plagiochasma crenulatum*, but differs in that the ventral scales reach the margin of the thallus. This Mexican form presents some variations in external appearance, and an examination of structure offers some details not covered by LEITGEB, so that it has seemed worth while to give a description of the external appearance, an account of the internal structure of the thallus and of the formation of rhizoids, a complete description of the development of the air cavities of the archegonial receptacle, and an addition to the history of development following fertilization.

Vegetative body

The thallus (fig. 1) is dorsiventral, dichotomously lobed and branched, with no groove on the dorsal side, but on the ventral side (fig. 2) a thickened median region, covered thickly with scales and rhizoids. The upper surface is green, showing purple at the edges; the ventral is light along the central region, with broad purple wings; the scales are in two rows, extending laterally from the median line, arranged alternately; they are attached along the posterior edge, lapping over those in front, and are shaped like the half of a crescent and have one or two appendages, the scales being purple and the appendages hyaline (fig. 3). Two appendages seem not to have been reported before.

The first rhizoids appear behind the first three or four pairs of scales and then occur in great abundance between the scales. They are of two kinds, pegged and smooth, the pegged appearing in bunches. They are deformed at the ends (fig. 4), but are not branched. The smooth rhizoids vary greatly in size, and frequently within the larger, smaller ones are produced (fig. 5); whether this occurs only when the larger are injured could not be determined, as most of the rhizoids were broken in collecting. The exterior rhizoids, having heavy walls, appear much older than those inside. This phenomenon is not uncommon in liverworts, often appearing in *Marchantia*, in which pegged rhizoids sometimes occur within plain ones. DIXON (**9**) also reports rhizoids within rhizoids in *Lunularia cruciata*, *Dumortiera hirsuta*, *Conocephalus conicus*, *Anthoceros punctatus*, and *Marchantia polymorpha*. WEINERT (**17**), in a study of *Marchantia* and *Conocephalus*, says "lost rhizoids are not regenerated, but other epidermal cells grow out into new rhizoids," which is plainly the case here. A new cell or several new cells just above the layer of old rhizoids push down into the cavity of the old rhizoid.

Structure

The thallus shows the usual differentiation into thin wings of spongy tissue and a thick median region composed of close colorless tissue below and spongy chlorophyllose tissue above, the latter often occupying almost all of the thallus. The cavities have no regular chlorophyllose filaments, although single cells frequently project into them (figs. 11 and 16). In these respects *Aytonia* differs markedly from *Marchantia*, *Fegatella*, and *Targionia*.

The close tissue of the thallus is generally infested with intracellular fungi (fig. 6), filamentous, non-septate, not contracted in passing through the walls, branching irregularly, and forming sporelike bodies occasionally as vesicles within a swollen part of the main filament or in short branches. One naturally questions the origin of the fungi and the relation existing between them and the liverworts. CAVERS (**6**) reports fungi in many liverworts, and says they are nearly always related to the soil, always occurring with humus; then he finds them traversing the rhizoids and extending into the lowest layers of the compact tissue; he thinks the fungus-bearing plants are larger and thicker than others. GOLENKIN (**12**) reports "endotropic mycorhiza" in five liverworts, and thinks they are "for storing water." NĚMEC (**15**) implies the entrance of the fungi through the rhizoids, and describes a pseudoparenchyma formed by them on the wall of the cells of the thallus. GARJEANNE (**10**) also finds them entering through the rhizoids and thinks the relation may be accidental, a case of true parasitism, or a case of true symbiosis. Miss CLAPP (**7**) is the only one who has traced the history of the infection back to the very early stages of the sporeling. She finds fungi in as young a plant as the 4-celled stage, and states that development is hastened by the presence of the fungus. According to her, infection of rhizoids occurs from the thallus. I am inclined to think that both methods of infection of the rhizoids may happen; that the plant may become infected at any stage of development; when considerable mycelium has developed, the hyphae may grow out through the rhizoids; and when the infection occurs late the hyphae may enter through the rhizoids. In my material the fungi are only in the older parts of the thallus, and when they occur in rhizoids seem to be always growing toward the thallus (fig. 7); but in living material of *Marchantia* and *Fegatella* I have found the hyphae passing out through the rhizoids. I find no "pseudoparenchyma" or balling up of hyphae, described by NĚMEC.

Cells secreting mucilage and resinous oils appear singly in thallus, receptacles, and scales; sometimes there is a layer of enlarged mucilage cells on the lower surface. Mucilage hairs are common about the receptacles and in the antheridial cavities. The middle lamella of the radial walls of the epidermal cells shows a tendency to become mucilaginous. Pitted cells occur in both the compact and loose tissue; they are not elongated more than other cells (fig. 8).

Development

Growth takes place by divisions of a wedge-shaped apical cell (fig. 9), as described by LEITGEB. From the lower segments scales are formed almost immediately, but rhizoids not until later. The epidermal cell giving rise to a rhizoid is often larger than the resulting rhizoid, forming a bulbous base. Division in the lower part of the thallus is more rapid than in the upper, indicated by the pulling backward of the bottoms of the air cavities. LEITGEB'S theory of the origin of air cavities, as due to depressions in the surface and the upgrowth of adjacent parts, was combated by BARNES and LAND (**1**) and by PETSCH (**16**), but was upheld by Miss BLACK (**2**) in regard to *Riccia Frostii*. My material leaves no doubt as to their schizogenous origin (fig. 10). LEITGEB thought the spaces in *Plagiochasma* became partitioned by the outgrowth of cell plates. CAMPBELL (**4**) also says that the original air chambers become divided by the development of partial diaphragms into secondary chambers. The cavities at first are always deep and narrow, as noted by BARNES and LAND, but they soon become wide, irregular chambers by the stretching and tearing of tissues between neighboring chambers, as shown by the irregularity of the resulting surfaces and by torn cells, the tearing being due to the differences in tension between the upper and lower parts of the thallus. This leaves projecting plates of cells, appearing as filaments in section, which LEITGEB and CAMPBELL interpreted as new growth dividing the original chambers. Perhaps these plates add to their length by further growth. In fig. 11 projecting filaments *a* and *b* are attached in the next section.

The receptacles are formed in a direct line behind the growing point. LEITGEB noted that they are not complete branches of the thallus, but only dorsal outgrowths. As the apical cell goes on dividing, the receptacles come to stand on the back of the thallus in a row along the median line, the antheridial appearing first, the archegonial later. Occasionally antheridial receptacles are again formed after the archegonial. Several rows of scales and mucilage hairs, dorsal outgrowths also, surround both receptacles (fig. 12).

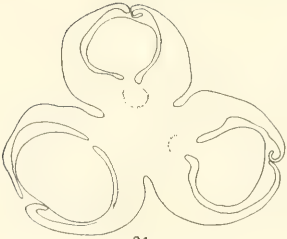
The first indication of a receptacle is a slight elevation caused by a rapid division of the cells. My material has no early stages of the antheridial receptacle; when mature it is without a stalk and does not rise much above the surface of the thallus (fig. 12); it is cordate in shape, with the notch toward the growing point (fig. 1). The surface is markedly papillate, caused by the upward growth of tissue about the antheridia, which agrees with LEITGEB'S description. The deep cavities in which the antheridia stand, open to the surface by simple air pores, resembling those of the thallus except that at times there is greater projection (fig. 13). The archegonial receptacle appears in an early stage (fig. 14) as a small, bulbous outgrowth of compact, rapidly dividing cells in which are numerous chloroplasts containing starch. The cells of the under part divide radially as well as tangentially, with such regularity that a row of cells can be followed for some distance, dividing into two rows, and these two later dividing again. In the lower portion the divisions are tangential only, but the cells increase both in length and in numbers. The lower part therefore forms the stalk, while the upper part continues bulbous. The cells of the thallus below the receptacle remain compact, while those on each side form porous tissue, growing rapidly and arching up around, so that the receptacle stands in a slight depression (fig. 12) until after the archegonia are mature. Fertilization probably takes place very easily. Later the stalk elongates and lifts the receptacle above the surface; it may attain the length of 3 or 4 mm.

Air cavities appear early in the developing receptacle, starting about the same time as the archegonia, the origin being like that of the cavities of the thallus. The split occurs first at the juncture of epidermal and hypodermal cells (fig. 10), between cells of different segments of the apical cell, cells derived from the same segment remaining attached for a longer time. The split may extend almost immediately to the surface (fig. 15) or be delayed until the formation of the pore (fig. 16). The structure of the pore is more elaborate than in cavities of the thallus, as noted by CAMPBELL (3) in archegonial receptacles in general. The cells destined to form the margin become evident by an increase in size (fig. 15); segments are cut off from the inner side, generally by oblique walls (fig. 17); more divisions occur until 3–5 rows of cells form a ring extending down into the enlarging cavity (fig. 18); then segments are cut off from the outer part of the cells of the margin, walls being again oblique, followed by another division, so that a projecting ring of cells three layers thick is formed (fig. 19). The cells of the last row shrink and become highly cutinized, giving a beaklike effect in section (fig. 20), as noted by DEUTSCH (8) in *Targionia*. I find no such thickening of the walls as LEITGEB shows in surface view, but only such an effect as appears in fig. 21, taken from the older part of the thallus. Of course my material may have grown under conditions entirely different from his. With the increase in size of the cavity the pore spreads and becomes a barrel-like opening. The development is not complete until fertilization has taken place and the receptacle has increased greatly in size.

Sex organs

The development of the antheridium is probably such as is general in the Marchantiaceae, but my material has no early stages. Occasionally the antheridium has a long stalk; less frequently the upper end is beaked; but the usual form is short-stalked and conical. About the antheridium are glandular hairs, and among the cells forming the wall of the pit are mucilage cells, the secretions from which probably assist the dispersal of the sperms. The antheridia are produced in acropetal succession, so that the younger are toward the apex (fig. 12).

The archegonia are reported by CAMPBELL (5) to be 3 or 4 in number, the one to the rear developing first, the lateral ones next, and the one in front last. My material confirms the statement in general, but 5 archegonia may occur. They begin development near the top of the receptacle (fig. 14), but are carried under by the rapid growth of the tissue above (fig. 12). The stages of development that appear indicate the sequence usually given. In 1897 GAYET (11) claimed that he had demonstrated in *Marchantia*, *Preissia*, and other Hepaticae, that division in the cover cell by intersecting quadrant walls does not take place until a late period, and that before it occurs repeated segments are cut off its lower face, which add to the length of the neck. Figs. 24, 26, and 27 leave no room for doubt that in *Aytonia* a quadrant division occurs early in the cover cell, and that the neck lengthens by divisions lower down. The neck cells divide simultaneously until 8 cells are formed (fig. 27), showing greater regularity than is commonly reported. The egg has a large nucleus but no "receptive spot"; it fills the cavity of the venter up to the time that fertilization is to take place; then the cells forming the wall of the venter divide rapidly, parallel to the surface of the egg; at the same time there is an increase in the size of the cavity or



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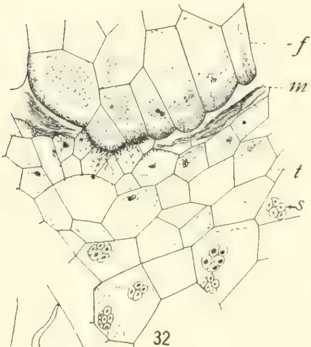

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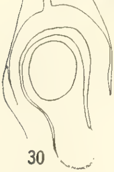

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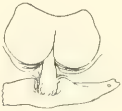

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FIGS. 30–33.—Fig. 30, vertical section of a young sporogonium within the calyptra, ×132; fig. 31, transverse section of an archegonial receptacle, cutting obliquely through three sporogonia within their involucres which show the vertical opening; fig. 32, section through the region of contact of foot and thallus (*f*, foot; *t*, thallus; *m*, mucilaginous remains; *s*, starch), ×667; fig. 33, sketch of archegonial receptacle indicating the position of the sporogonia.

else the egg shrinks, so that there is considerable space between the egg and the upper part of the venter. The egg then generally contains large, perfectly spherical globules that stain deeply red with safranin; the shape suggests a fatty food substance. The breaking down of the canal cells leaves the egg invested with a great amount of striated mucilaginous substance which often fills more than half the venter (fig. 28). When the first division of the embryo occurs, all the cells of the venter have divided. Growth continues in the stalk, the venter, and the lower part of the neck until an extraordinary amount of tissue is developed about the embryo and the neck is greatly elongated, curving up above the cushion of the receptacle (fig. 29).

Sporogonium

The embryo develops in the usual way, beginning with a transverse wall. At first the seta appears as of considerable length when compared to the whole body (fig. 30), but later the capsule and foot develop more rapidly, so that the seta comes to be inconspicuous (figs. 31 and 33). The foot is well developed as an absorbing organ, the cells next to the thallus being large and slightly rhizoidal in shape. Just within the walls of these cells and of the adjoining cells of the thallus there is a granular deposition, probably related to the passage of nutrient material from the thallus to the parasitic sporogonium. The cells of the thallus respond to the invasion of the foot by dividing, as is shown by the cells nearest the foot being smaller than elsewhere. As the cells break down, a mucilaginous deposit remains, marking the limit between foot and thallus (fig. 32). As the sporogonium matures, the starch contained in the cells of the thallus near the foot disappears, and in all the cells of the receptacle it is scantier than in earlier stages.

The history of the development of the capsule and of the involucre, resembling the valves of a clam shell, has been described by Leitgeb. It might be added that a few cells at the base of the capsule remain sterile, but there is no regular elaterophore.

Summary

1. Two appendages may be present on the ventral scales.

2. Rhizoids are absent among the first pairs of ventral scales.

3. Old rhizoids are often replaced by new ones that form within them.

4. Fungi are prevalent in the compact tissue of the thallus. They enter through the rhizoids and may also pass out through them. No "pseudoparenchyma" was found.

5. The secretion of mucilage seems to have nothing to do with the "protection" of the growing point, but to be most pronounced about the egg and the antheridia.

6. Pitted cells show no tendency to become trachea-like.

7. The origin of air chambers in the thallus and receptacles is schizogenous. The horizontal increase in the size of the chambers is due to a tearing of the tissues. The pore of the chambers of the thallus and of the antheridial receptacle is simple, but that of the archegonial receptacle has an elaborate margin.

8. The development of the sex organs follows the Marchantiales type. The archegonia form early in the history of the receptacle and parallel the increase in size of the receptacle by great increase in length of the neck. Following fertilization an exceedingly massive venter is developed about the embryo.

9. Five archegonia may begin to develop on one receptacle, but no more than three come to maturity.

10. The condition of the cells of the foot and of the adjacent parts of the thallus indicate the parasitic nature of the sporogonium. No elaterophore appears.

Most cordial thanks are due Dr. W. J. G. Land, who furnished the material for this study and followed the work with interest and encouragement.

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EXPLANATION OF PLATES I–IV

Fig. 1.—Sketch of monoecious thallus, dorsal view.

Fig. 2.—Sketch of thallus, ventral view.

Fig. 3.—Sketch of ventral scales with appendages.

Fig. 4.—Sketch of rhizoids; ×470.

Fig. 5.—Longitudinal section of an old rhizoid with a new one developing within.

Fig. 6.—Section of compact tissue by the thallus with fungal hyphae passing through the walls and destroying the protoplasm; ×450.

Fig. 7.—Fungal hyphae entering a thallus through a rhizoid.

Fig. 8.—Surface view of a pitted cell; ×687.

Fig. 9.—Vertical longitudinal section of a thallus through the apical cell, showing the origin of ventral scales (*s*) and air chambers (*a* and *b*); ×375.

FIG. 10.—Vertical section of developing air cavities in an archegonial receptacle; ×750.

FIG. 11.—Vertical section of an air pore of the thallus; ×667.

FIG. 12.—Vertical longitudinal section of a thallus with two archegonial receptacles and one antheridial.

FIG. 13.—Vertical section of a pore in an antheridial receptacle.

FIG. 14.—Vertical section of an early stage of an archegonial receptacle; ×67.

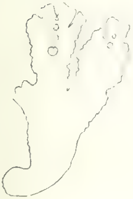
FIGS. 15–20.—Stages in the development of an air pore of the archegonial receptacle.

FIG. 21.—Surface view of an air pore of the thallus.

FIGS. 22–27.—Stages in the development of the archegonium before the formation of the egg; fig. 25 shows also the origin of a scale and of the involucre (*i*).

FIG. 28.—Vertical section of a venter after fertilization; *n*, nucleus; *g*, spherical globule; ×667.

FIG. 29.—Vertical section of an archegonium inclosing a young embryo; ×470.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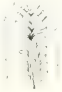
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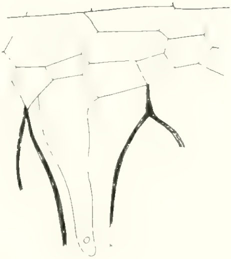
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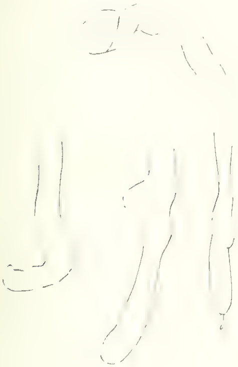
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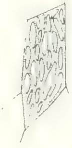
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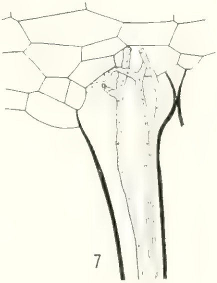
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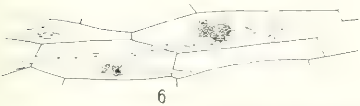
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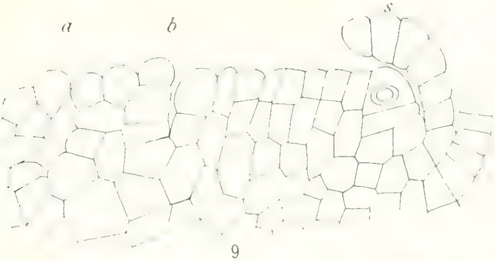

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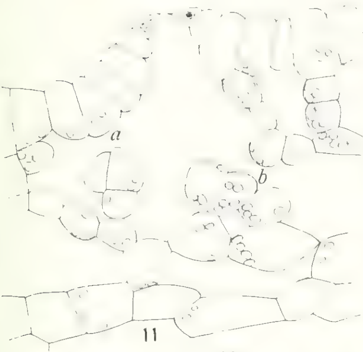

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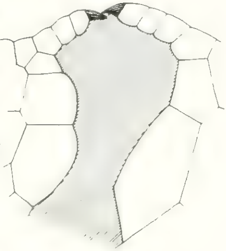

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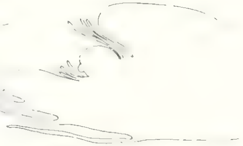

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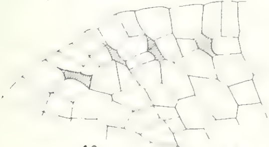

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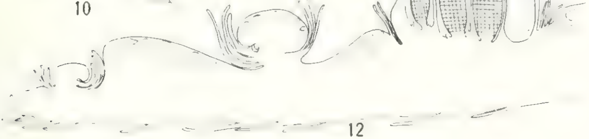

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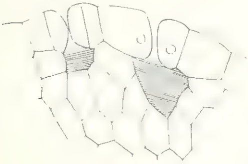

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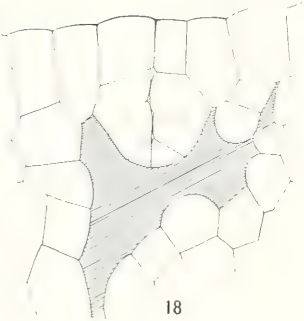

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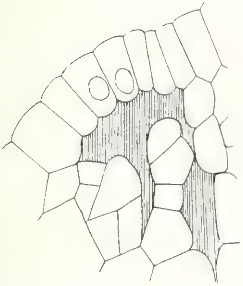

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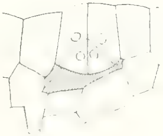

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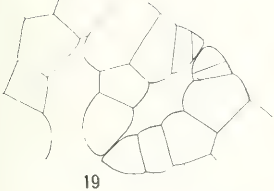

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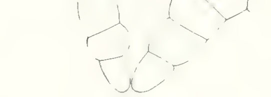

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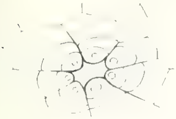

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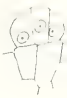

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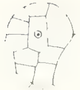

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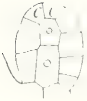

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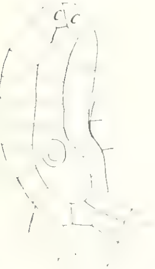

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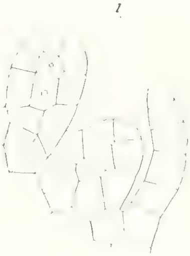

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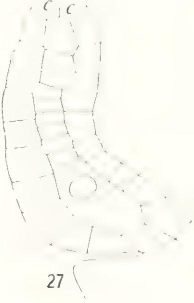

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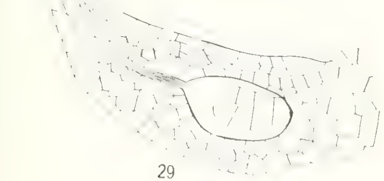

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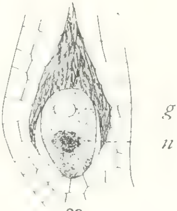

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STARR on AYTONIA

THE DEVELOPMENT OF THE CONCEPTACLE IN FUCUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 212

(WITH PLATES XIV–XVII)

MABEL LEWIS ROE

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THE DEVELOPMENT OF THE CONCEPTACLE IN FUCUS

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(WITH PLATES XIV–XVII)

Introduction

When engaged upon a study of *Splachnidium rugosum*, the writer was forced to work out a new theory for the development of the conceptacle, since methods previously described did not offer sufficient explanation for what was found in *Splachnidium*. It seemed probable that such might also be the case in more advanced members of the Fucaceae, particularly *Fucus*, and it was with this in mind that a re-examination of this well-known form was undertaken. The results have proved interesting enough to warrant the reinvestigation.

Material and methods

For the material the writer is greatly indebted to Professor CHARLES J. CHAMBERLAIN, who had at his disposal paraffin cakes of young tips of *Fucus edentatus* Delapyl. and Kjellman (*F. furcatus* Agardh et al.) and of *Fucus vesiculosus* L., of which he had also mature tips containing ripe conceptacles. In addition, he furnished the writer entire plants of *Splachnidium rugosum* Grev., collected by him at Glen Cairn, near Cape Town, South Africa. To Professor JOHN M. COULTER the writer is indebted for material of *Fucus serratus* L., which was collected at Pictou, N.S., by Mr. J. CRERAR MCDONALD, at the request of Professor W. G. FARLOW. All the material, except that used for fig. 20, was cut 5–8 μ in thickness, parallel to the long axis and at right angles to the flat surface of the thallus. Material for fig. 20 was cut 15 μ thick. The sections were stained with Haidenhain's iron alum haematoxylin and given a light touch before mounting with Grübler's Lichtgrün dissolved in equal parts of absolute alcohol and clove oil. The former stain brought out nuclear and cytoplasmic detail, while the latter gave in sharp relief all cell walls and layers of mucilage.

JUN 13 1921

Previous work

The first attempt at detailed, histological description of the origin of the conceptacle was that of KÜTZING in 1843 (**13**). He speaks there (p. 92) of the appearance on the surface of the thallus of small circular depressions, "Fasergrübchen" (cryptostomata), which have a raised border and contain finely septate ("gegliederte") hairs. He says that in their structure they resemble conceptacles ("Hüllenfrüchte") and seem to have a certain relation to them, although present in certain forms which do not possess conceptacles. Moreover, they appear on sterile as well as on fertile plants. He reports them sometimes lacking on *Fucus vesiculosus*, although usually present. He offers an ingenious explanation of the use of hairpits to the plant, describing a fusion of hairs developing a new plantlet as an adventitious shoot upon the parent.

He calls the conceptacle a closed-over sorus ("eingestülpter Sorus") and says (p. 98) that the layer forming the inner wall of the cavity is nothing other than a slight modification of the superficial layer of the cortex which becomes covered over and bears antheridia and paraphyses ("Samen und Nebenfäden trägt"). This cover conforms in structure to that of the superficial layer ("Rindenschicht") of the thallus.

AGARDH in 1848 (**2**) describes in the Fucoideae (p. 2) superficial cells prolonged into hairs, which project freely beyond the surface of the frond, either scattered or massed in a small bundle; this bundle is bounded by an elevated margin of adjacent cells. Such a body he calls a "cryptostomatum." The conceptacles ("scaphidia") he describes as suspended in the anastomosing filaments in the interior of the thallus, and hollowed out beneath the surface (p. 181). In the same place he mentions the occurrence of hairpits ("cryptae minutae") on sterile portions of the frond which are analagous to conceptacles on fertile parts ("scaphidiis in parte fertili analogae"), but that such "crypts" are often lacking. He describes a thin membrane in the early stage covering the mouth of the cavity which is a portion of the superficial layer ("quae tantum pars strati superficialis est"), but that the mouth of the cavity is later always open ("sed demum eadum semper evadunt ostiolo hiantia").

SACHS in 1875 (**26**) says (p. 227) of the conceptacle of the Fucaceae:

> "These conceptacles are not formed in the interior of the tissue, but as depressions in the surface which become walled in by the surrounding tissue, and so overgrown that at length only a narrow channel remains, opening outward. The layer of cells which clothes the hollow is thus a continuation of the external epidermal layer of the thallus, and since the filaments which produce the antheridia and oogonia sprout from these, these latter are, morphologically, trichomes."

In the same year REINKE (**21**) published an account of *Fucus vesiculosus*, followed a year later by a much more inclusive record of investigations (**22**) of a large number of closely related genera. In each paper he discusses the origin and development of both hairpits ("Fasergrübchen") and conceptacles. On p. 337 he gives the details. The first indication of a pit is noticed on the lip close to the growing point, where a few neighboring epidermal cells separate themselves from other tissue (comparable to formation of resin ducts in conifers) and form between them an intercellular space filled with mucilaginous substance. Also the cortical cells, just beneath, are involved in this separation, and the cavity thus developed widens into flask-form, wherein the original meristematic cells take on the usual character of the border cells. These border cells develop first into papillae, and by subsequent division into hairs which project beyond the mouth of the pit.

The conceptacle is regarded by REINKE as a structure comparable to the flower of angiosperms, and is a metamorphosed thallus apex and branches. In this metamorphosis hairpits are changed into conceptacles (p. 338). The details of development are about as in the hairpit (p. 339). On the exterior the cavity is rendered firm by the epidermis and a few layers of cortical tissue. The epidermis stops short at each side of the ostiole; the walls are formed by small, nearly isodiametric cells corresponding to the cortex. Hairs spring from the base of the cavity and project beyond the ostiole. Because of the place of origin (close to the growing point) and similarity in method of development (separation of groups of cells from adjacent external cells, etc.), he considers the conceptacle the homologue of the hairpit (p. 340), and that up to a certain stage there is no recognizable difference between the two ("auf diesem Stufe giebt es keinen erkennbaren Unterschied zwischen Fasergrübchen und Conceptaculum; wir haben es mit der Erscheinung zu thun dass ein der Anlage nach identisches Organ sich in einem Fall zu vegetativen, im anderen Fall zu reproductiver Thätigkeit entwickelt").

The next important contribution to this subject was the paper by BOWER in 1880 (**6**), whose work has been generally accepted since. In his introduction he gives a very brief résumé of previous work, given in the foregoing in greater detail. He first describes the development of the conceptacle and then compares it with that of the hairpit, which he regards "as an incomplete sexual conceptacle" (p. 37). He agrees with ROSTAFINSKI (**25**) as to behavior of segments of the apical cell, namely, that each segment divides first by a wall parallel to its free surface; the outer cell again divides in two planes at right angles to each other and to the first wall, thus giving four cells which may repeat the same method of segmentation as the original segment. A slight modification of this behavior initiates a conceptacle (or hairpit). Upon completion of a linear series of cells, activity in horizontal division ceases, leaving the terminal ("initial") cell in a depression as surrounding tissue continues active growth. This "initial" first loses its internal tension and later shrinks back against the basal segment beneath. Later segments of this basal portion (cortical in origin) line the bottom of the cavity, whereas the sides are derived from the limiting tissue (external layer) and from subjacent cortical tissue. This is the method for *Fucus*.

In *Himanthalia* (pp. 46, 48), the lining of the cavity is derived entirely from limiting tissue. In *Halidrys* he finds (p. 45) that

> "The basal cell divides at first by walls strongly inclined to one another. The cells thus produced do not at any early stage divide by walls parallel to the surface of the cavity. The result is that the conceptacle usually appears . . . . as though lined by a layer of cells continuous with the limiting layer; but as part, at least, of this tissue is derived from the basal cell, this conclusion is inadmissible. Meanwhile the initial cell (or group of cells) has been completely thrown off by the swelling of the wall dividing it from the basal cell. Later, as in other plants of this group, the cells of the lining tissue put forth papillae which develop further into hairs."

BOWER pays considerable attention to the "central column."

> "The change of the substance filling the cavity has advanced so far that the unaltered portion immediately surrounding the remnants of the initial cell forms a central column of irregular outline. This stretches from the basal cell to the neck of the conceptacle, and is connected with the walls of the conceptacle by thin strings, which, like itself, have remained as yet unchanged" (p. 40).

He offers as an explanation that there are three substances present: (*a*) a swollen form of cell wall, which is not true cellulose, but is similar to the central portion of older cell walls of the tissue of the larger tangles; (*b*) a substance coincident with mucilage; and (*c*) a substance akin to cuticle. Later the connecting strings are ruptured.

In conclusion (p. 47), BOWER states that

> "In all the cases described, the formation of the conceptacle is preceded by the decay of one or more cells which occupy a central position with regard to the changes which follow. The number of the cells thus removed is various, and the manner of their distribution is not constant. . . . . A point which is not so obvious, but which appears of similar constancy, is that the cell or cells which decay are in all cases members of a linear series. It depends upon the activity of division, in a direction tangential to the surface of the thallus, how this series is characterized; whether, as in *Fucus*, where the division is slow and even ceases, the apical cell of the series hangs behind the surrounding tissue; or whether, as in *Himanthalia*, where the division is often repeated, the series is elongated, and, protruding beyond the surface of the thallus, is called a hair"; and (p. 48): "This variation in activity of tangential division accounts for the want of uniformity in number of the cells thrown off in different species, and even in the same species. . . . . The differences in mode of development (in the early stages at least) depend upon the difference in activity of tangential division of the cells of the central series."

BOWER regards the hairpit as an incomplete conceptacle, but that "The homology of the two structures is so clearly proved that I shall be justified in proposing . . . . the name 'neutral conceptacle' (p. 44). I think it is important to convey at once the relation which appears to exist between them and the true conceptacle."

VALIANTE in 1883 (**29**) describes the method of development for *Cystoseira* and related genera. Primordia develop in the apical grooves of ultimate branches, a hollow being formed just as in early stages of hairpits. In either case, one or more peripheral cells at some point on the inner surface of the groove lose their power to increase in size and are left in a small cavity by active growth of surrounding cells. The initial (or initials) do not disintegrate, but by transverse divisions grow into hairs which project beyond the mouth of the conceptacle. He says he can find no traces, in his material, of decay of elements preceding formation of a cavity, as described by BOWER for *Fucus*. He finds hairpits developed among the conceptacles at apices of fruiting branches, but that in such cases the cavity is less pronounced than usual. He considers these a convincing proof of the close relationship between hairpits and conceptacles.

OLTMANNS (**19, 20**) considers that the method as described by BOWER is essentially constant for all the Fucaceae, with very unimportant exceptions, namely, that the cells abutting on the "initial" have the chief work in organizing a conceptacle, sharing this with the "basal" cell, whereas the "initial" seldom remains intact, but either disintegrates or else grows into a hair ("überall kommt ihr zweifellos irgend eine nennenswerthe Funktion nicht zu"; **20,** p. 516). OLTMANNS regards the conceptacles and hairpits as homologous structures, but takes exception to BOWER'S conclusion that hairpits are "neutral (sterile) conceptacles," for he believes that the hairpits have become sexual (**19,** p. 82).

BARTON in 1891 (**3**) made the next contribution, in her study of *Turbinaria*. She found the conceptacles and hairpits developed in essentially the same way as described by BOWER, VALIANTE, and OLTMANNS, the "initial" cell being persistent. "The initial cell divides longitudinally, and both cells, after again dividing transversely near the top, grow into hairs, the upper division of the initial forming the swollen base of the mature filament. These filaments fill the fully grown conceptacle" (p. 224).

She objects decidedly to BOWER'S terms "neutral" and "sterile conceptacle" as "conveying an idea of abortive growth" (p. 223). Her view is that the two bodies are of "equal antiquity" and "a later development in the ancestors of the Fucaceae than the reproductive organs." "I consider neither form a development of the other, and the fact that one conceptacle contains reproductive organs, the other nothing but paraphyses, is an interesting point, but does not bear on the phylogenetic history of the conceptacles themselves." She then proposes the term "vegetative conceptacle" as meaning "those cavities in the thallus which have been developed only in a vegetative direction." In commenting on BARTON'S theory, MURRAY (**18**) says (p. 60) "I know so little about the ancestors of the Fucaceae, that I must be content with a respectiful attitude toward this statement."

In their research upon *Splachnidium*, MITCHELL and WHITTING in 1892 (**17**) find that the conceptacle is developed by alteration of one of the epidermal cells, close to the apical cell, which becomes peculiarly modified but is inconsequential; however, they consider it the homologue of BOWER'S initial, although it takes no further part in developing the conceptacle, the real work falling to the neighboring cells.

In interesting connection with work on the conceptacle is that on hairtufts and sori in groups outside the Fucales.

MITCHELL in 1893, in a study of *Hydroclathrus* (**16**), finds groups of hairs analagous to "Fasergrübchen" (cryptostomata) of the Fucaceae. In development they certainly resemble the conceptacles, and her description recalls REINKE'S work. She speaks of an isolated cell or group of cells which become separated from surrounding epidermal tissue. Transverse division results in formation of hairs from these isolated cells. Meanwhile growth of the thallus leaves the isolated portion in a slight depression. Later, the sporangia which are developed in close proximity around the hairpits mature, and after liberation of the spores the sporangial walls disappear and the basal cells originate new growth. Finally, all the sporangia disappear and these hairtufts, with central depressions, are left scattered over the thallus, persisting throughout the life of the plant. MITCHELL concludes that although not a true conceptacle, such as in the Fucaceae, yet the growth of both hairs and reproductive organs is initiated by alteration in form and subsequent division of epidermal cells which might with truth be called "initial" cells. "My observations do not exclude the possibility of the initiative being taken by a small group of initial cells dividing simultaneously, instead of a single one."

MURRAY (**18**) states that the development of both cryptostomata and conceptacles follow the method described by BOWER. He quotes BOWER and then adds "These words appear to me to be the true guide of those who investigate the development of such bodies" (p. 60). In conclusion he states that comparison of the conceptacle of the Fucaceae with cryptostomata either in the heart of a sorus, as in *Adenocystis* and *Hydroclathrus*, or apart from the sorus, as in *Alaria* and *Sacchorhiza*, and with the situation in *Asperococcus*, in Cutleriaceae and in Dictyotaceae "points very significantly to a possible origin of cryptostomata. I anticipate, from further research into the development of these bodies, evidence that may enable us to dispense with the ancestors of the Fucaceae, of which, however, I would speak with respect" (p. 63).

BARTON in 1898, in a study of *Soranthera* (**4**), *Colpomenia*, and *Chnoospora* (**5**), found an interesting development of reproductive bodies in connection with cryptostomata, giving further evidence along the line already noted by MURRAY in his study of *Adenocystis*. In each case a saucer-like depression initiated the central portion of a cryptostoma and later of a sorus with reproductive sacs, whether these latter be plurilocular or unilocular. The important fact is that both portions of the sorus always originate as modifications of the superficial layer of the vegetative body.

HOLTZ in 1903, in his work with *Pelvetia* (**11**), describes a method of development little different in essentials from BOWER's. Several epidermal ("initial"?) cells cut off a series of segments beneath to form a sort of pad of meristematic cells; then the external portions break down more or less, forming a cavity. The inner portions line the base of the cavity and later give rise to paraphyses and sex organs. Meanwhile unaffected epidermal cells continue division and cut off basal segments which become part of the cortex. "This new cortical growth stops abruptly at the conceptacle. In this way the cavity is deepened and a neck is formed, this neck being composed of epidermis-like cells. Original cortical rows are slightly deflected around the forming cavity, but later become deeply invaginated and thus aid in deepening the conceptacle" (pp. 35–36).

SIMONS in 1906, in a study of *Sargassum* (**27**), claims a development of conceptacle "At variance with all the prominent characteristics . . . . of the conceptacle as described by BOWER." The initial does not break down, but is an active cell producing the entire conceptacle. Adjacent cortical tissue is in no way involved in the process (p. 169). "Both the conceptacles and cryptostomata originate in a single flask-shaped initial which develops the entire structure. The first division of the initial results in two unlike segments: a large lower cell which develops the walls of the conceptacle and cryptostoma; and an upper cell, the tongue cell, which either remains inactive, divides to form a short filament, or degenerates. The "initial" cell of BOWER is apparently the tongue cell, a product of the true initial cell. The conceptacle and cryptostoma are undoubtedly homologous structures. Every stage of development in both structures is the same, from the appearance of the similar initial cells to the development of paraphyses in the cryptostomata and sexual organs in the conceptacle" (p. 179).

Summary of literature

The various theories described in the foregoing seem to resolve themselves into three categories: (1) the conceptacle or hairpit is a slight modification of the external layer of the thallus; this theory was held by KÜTZING, SACHS, and LUERSSEN (**15**); (2) the conceptacle is a product of one or more initials, which do or do not disintegrate; their basal segments form the basal portion, whereas adjacent cortical tissue completes the sides of the structure; this theory was held by REINKE, BOWER, VALIANTE, OLTMANNS, BARTON, MITCHELL, WHITTING, MURRAY, HOLTZ, FALKENBURG (**7**), and others; (3) the conceptacle is a product of a single initial whose segments develop the entire body; this is the theory of SIMONS. All of these workers agree, more or less, that conceptacles and hairpits are homologous structures.

Description

In a study of three species of *Fucus* and of *Splachnidium rugosum* and a rather superficial examination of *Sargassum filipendula* and *Hormosira*, the writer found evidence which supports the early claim that the conceptacle is merely a slight modification of the original external layer of the thallus.

Following segmentation of the apical cell, in *Fucus*, some segment on the inner lip of the apical groove, close to the apical cell itself, ceases activity, and through failure to continue growth for a time is left in a depression by the active growth of abutting tissue. This inactive segment may then begin to break down in its external portion, without cutting off a transverse segment beneath to give a "basal cell" (figs. 1–4, 5*c*). Again, a basal segment may be cut off (figs. 5*b*, 6, 11–15), or even two segments may behave in a similar fashion (figs. 8, 9, 10). As the surrounding tissue comes to surpass the original inactive segment, the cells immediately abutting begin to break down in their external portions, as did the original segment (figs. 1 15). By continued involving of outer segments the cavity is gradually enlarged, and since the adjacent segments are always more active and therefore have greater turgor, the neck of the structure is narrow, whereas the basal part is broad.

Meanwhile, more and more of the outer portions of the cells involved break down, becoming mucilaginous and often showing several layers when stained with Lichtgrün (figs. 4, 6, 8, 10, 12, 14, 15). Ultimately, all except the portion immediately surrounding the nucleus having disintegrated, the original segments are left as mere lumps of tissue lining the cavity (figs. 6–15). *These basal portions never lose their meristematic activity*, but after a period of quiescence begin to put forth papillae as the cavity enlarges (figs. 16, 17). These papillae develop into hairs by basipetal segmentation, and finally become mature, multicellular, unbranched hairs which fill the cavity and project beyond the opening, out over the surface of the thallus (figs. 15–18).

Some time after maturity of the hairpit, these hairs are shed, and from the basal portions (*of the original segments*) new papillae put forth, this time to develop into hairs of a more delicate and fragile nature, often lost very shortly after (figs. 18, 19). Up to this stage there is no appreciable difference between hairpit and conceptacle. Moreover, further development merely offers a means of distinguishing antheridial from oogonial conceptacles.

if the species happens not to be monoecious. Immediately upon loss of the fragile hairs described in the foregoing, or from basal portions which failed to develop them, new papillae develop which by their subsequent behavior indicate whether antheridia or oogonia are to come. In the former case, segmentation may continue until an elaborately branched and complicated structure bearing numerous antheridia is formed. In case of formation of oogonia, the papillae segment once, cutting off the pedicels and oogonia proper (fig. 20). Further development up to blocking off of 8 eggs (fig. 20) is as already described by many writers (FARMER and WILLIAMS **9**, OLTMANNS **19** and **20**, STRASBURGER **28**, YAMANOUCHI **30**, SIMONS **27**, and others).

In *Splachnidium rugosum* the cavity is initiated by disintegration of an entire row of the thallus (and a terminal hair as well in some cases), pressure of active cells on each side reducing the disintegrating row to a long filiform structure which is left free subsequently by withdrawal of abutting cortical tissue in its effort to keep pace with active tissue toward the exterior. The abutting tissue at the external surface continues division and active growth, leaving the original initial row in a depression. Reproductive organs succeed a loss of hairs in the pit, just as in *Fucus*, except that here there are no evident antheridia or oogonia, but merely reproductive sacs.

Examination of *Hormosira* and *Sargassum* material seems to show the same behavior.

Discussion

It will be noted from the foregoing description that the *sex organs appear after maturity of the hairpit*, and, *moreover, they appear in the hairpit itself*. In other words, the hairpits and the conceptacles are certainly homologous structures, as already admitted by many, since one, *the hairpit, is the juvenile stage of the other, the conceptacle*. *All hairpits*, therefore, *are potentially capable of producing reproductive organs*.

Moreover, sex organs have actually been seen in hairpits, described by SIMONS (**27**) as a "peculiar condition" (p. 173). She finds papillae and stalked cells like those which precede male organs in a conceptacle or like the male organs themselves. She considers this condition a proof that hairpits are derived from the conceptacle (p. 174).

The writer is strongly inclined to the belief that those forms which are reported to possess hairpits only, with no evident connection to reproductive organs, will, upon further investigation, show closest relationship to reproductive activity, as already proved in research upon forms of Ectocarpaceae, Laminariaceae, Dictyotaceae, and Cutleriaceae. To the writer the resemblance of *Hydroclathrus* (**16**) to Fucaceae, where it is reported that after shedding sporangia basal cells originate new growth, is startling. In all the cases where similar sori are described, the origin is constantly from modification of the external layer, even when the writers themselves do not emphasize this fact. BOWER's description of *Halidrys* and *Himanthalia* (**6**) seems to the writer good evidence against his own theory, and the writer is inclined to the opinion that with technique as it is developed today, BOWER would find the conceptacle of *Fucus* of quite the same origin as in *Halidrys* and *Himanthalia*. Even as it stands, several of BOWER's drawings (pl. 5, figs. 2, 5, 11) show a possibility of interpretation quite like that of the writer.

Sections made by the writer of the same species of *Sargassum* cast some doubt on the explanation offered by SIMONS (**27**). Moreover, sections of young tips of *Fucus vesiculosus* give almost identical stages like those shown in her drawings of *Sargassum* (pl. 10, figs. 1, 3, 5, 6, 12, 17, etc.). However, sections of later stages show subsequent behavior to be quite different from that described by SIMONS, and to be as described by the writer for *Fucus*. It remains for further investigation to prove whether SIMONS is entirely accurate in her description. It seems to the writer that what SIMONS interprets as segments of the basal portion of the "initial" are really the basal portions of original segments of the apical cell, similar in nature and behavior to the "initial."

As to the various terms suggested, BOWER's "neutral conceptacle" ("sterile conceptacle") and BARTON's "vegetative conceptacle" are equally unhappy; if the body is a true conceptacle it cannot be "neutral," "sterile," or "vegetative." Since the modern trend is for simplicity rather than complexity, and since the hairpit is simply an early stage of the conceptacle, the writer is strongly inclined to reject both BOWER's and BARTON's terms and retain the simpler term "hairpit," an exact translation of the early German use of "Fasergrübchen." BOWER's "central column" is explained by examination of figs. 13–15. The old walls become mucilaginous and sometimes several layers of mucilage are seen between the meristematic portion and the "column."

Conclusion

There seems a clear line of advance from forms with continuous patches of hairs and reproductive organs, as *Nereocystis* and allied forms, to distinct sori, where the hairpit is the center of a reproductive group, as in some Ectocarpaceae, Laminariaceae, Cutleriaceae, and Dictyotaceae, to distinct conceptacles scattered over the entire vegetative body, as *Splachnidium*, *Hormosira*, etc., to a final distinct grouping at the apex of a shoot, as in *Fucus*, *Pelvetia*, and other Fucaceae, or on special side branches, as in *Sargassum*, *Turbinaria*, etc. Similar lines of advance are already commonly accepted for bryophytes and pteridophytes, in the one restricted to arrangement of sex organs, in the other to arrangement of sporangia. A striking parallel to *Fucus* is seen in *Corsinia*, *Riccia*, and allied forms, where, step for step, the development of the dorsal groove and subsequent appearance of sex organs (and even hairs) from the basal portion repeats the history of the development of the conceptacle of *Fucus* from an original segmentation of the apical cell. In this one sees a forceful illustration that plants again and again duplicate behavior in given conditions, even though in one case the plant may be a gametophyte and in the other a sporophyte.

Summary

1. The conceptacle originates as a slightly modified continuation of the external layer of the thallus, being segments of the apical cell whose basal portions are constantly meristematic and *never entirely breaking down*.

2. The hairpit is a juvenile stage of the conceptacle, the sex organs appearing in the same cavity as the mature hairs, but after their loss.

3. A distinct phylogenetic series is seen in advance from continuous patches of hairs and reproductive bodies, to scattered sori, to scattered conceptacles, and finally to apically placed conceptacles or to conceptacles on specially developed side branches. All of these structures originate through modification of the superficial layer of the thallus.

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EXPLANATION OF PLATES XIV–XVII

The drawings were all made with the aid of the Abbé camera and reduced one-half in reproduction. For figs. 1–18, the original magnification was 1050; for figs. 19–20, the original magnification was 535. Figs. 1–19 are of *Fucus edentatus;* fig. 20 is of *Fucus vesiculosus.*

Figs. 1*a*–20*a*.—Sketches to show topography.

Fig. 1.—The inactive segment of the apical cell; the external portion already shows a breaking down; adjacent cells also show inactivity in a similar way.

Figs. 2–4.—Further breaking down.

Fig. 5.—(*b*) Original segment which has cut off a basal segment; (original segment with no such segment cut off.

Figs. 6, 7.—Further stages of figs. 1–3.

Figs. 8–10. Two or more arrested segments.

Fig. 11.—Further stage of figs. 1–7; basal portions about nuclei very distinct.

Fig. 12.—Further breaking down; involving of abutting cells especially clear.

Figs. 13, 14.—Showing how old walls are attached to "central column."

FIG. 15.—Fragments in center due to disintegration of walls still attached to column in figs. 13, 14.

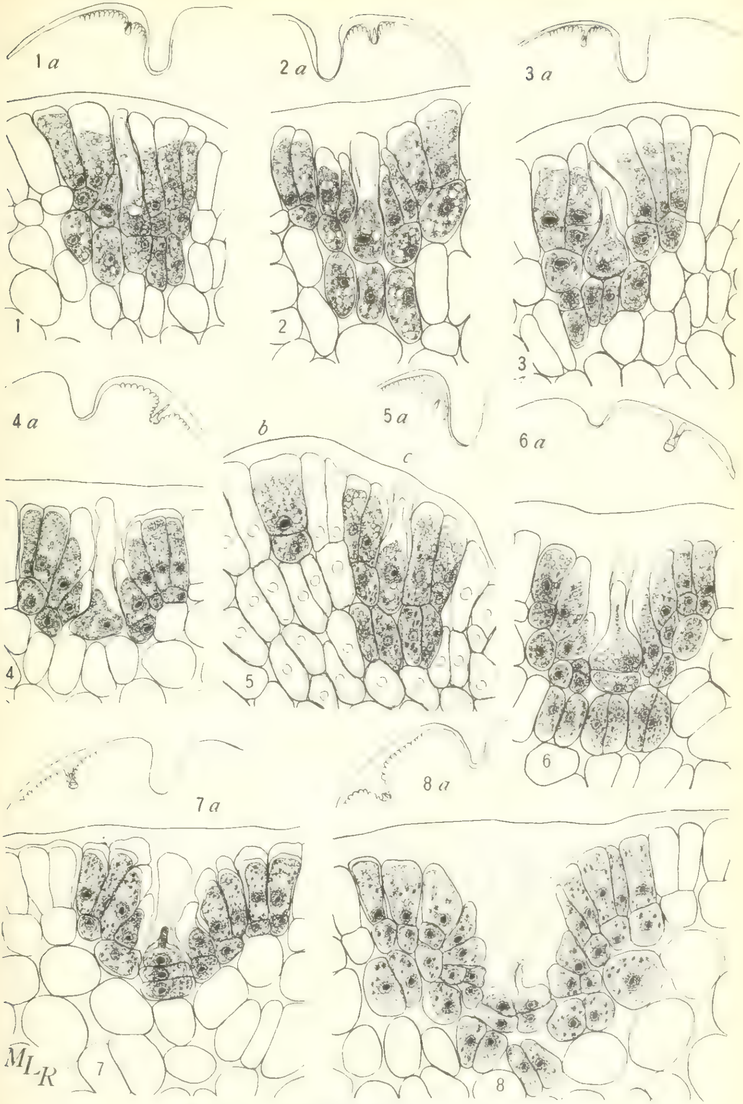
FIG. 16.—Young hairs developed from meristematic basal portions of original segments.

FIG. 17.—Older stage of same.

FIG. 18.—Mature hairpit; meristematic portions of segments seen on side with papillae.

FIG. 19.—Older pit; long hairs falling off; meristematic portions of segments putting forth papillae and delicate hairs.

FIG. 20.—Mature conceptacle; delicate hairs being shed; oogonia in all stages from one-celled to complete blocking off of 8 eggs, developed by papillation and subsequent segmentation from meristematic portions of original segments; old hairs still seen about the ostiole, as well as fragments scattered through the cavity.



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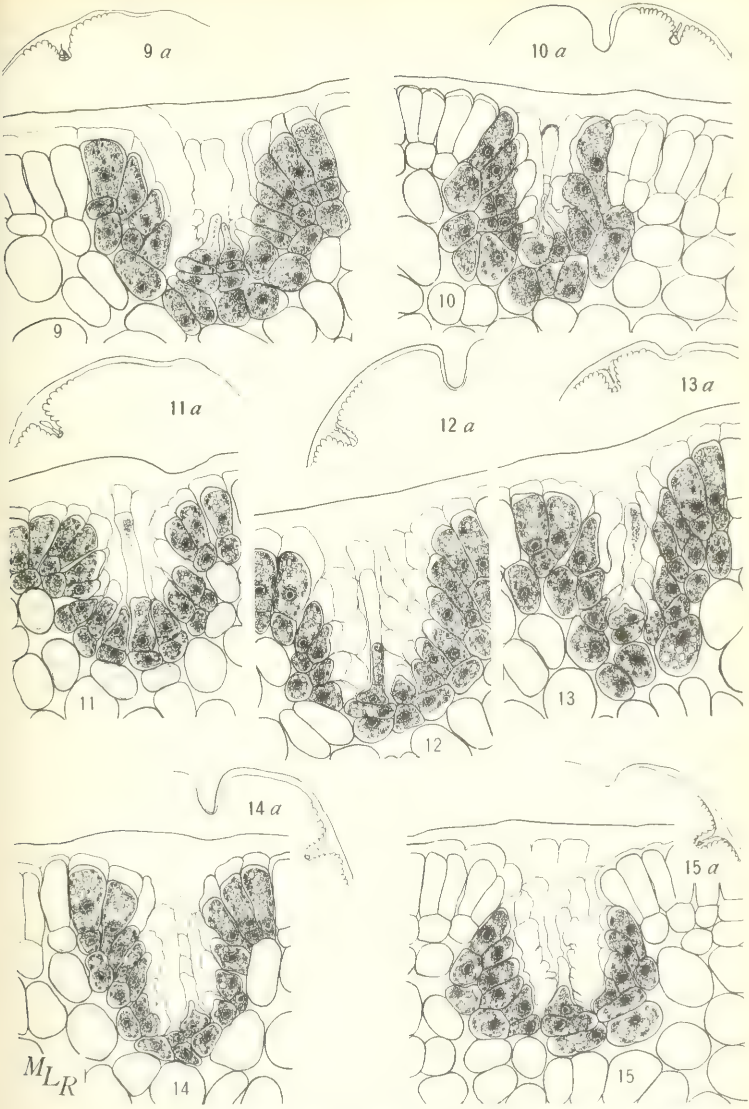

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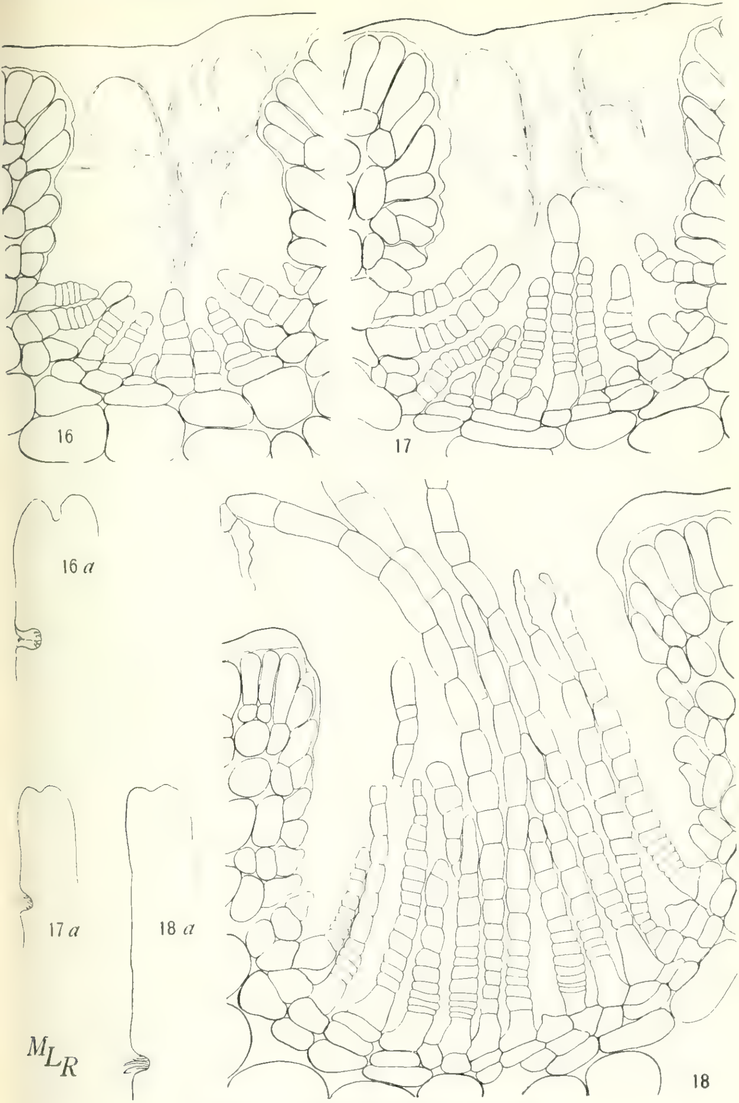

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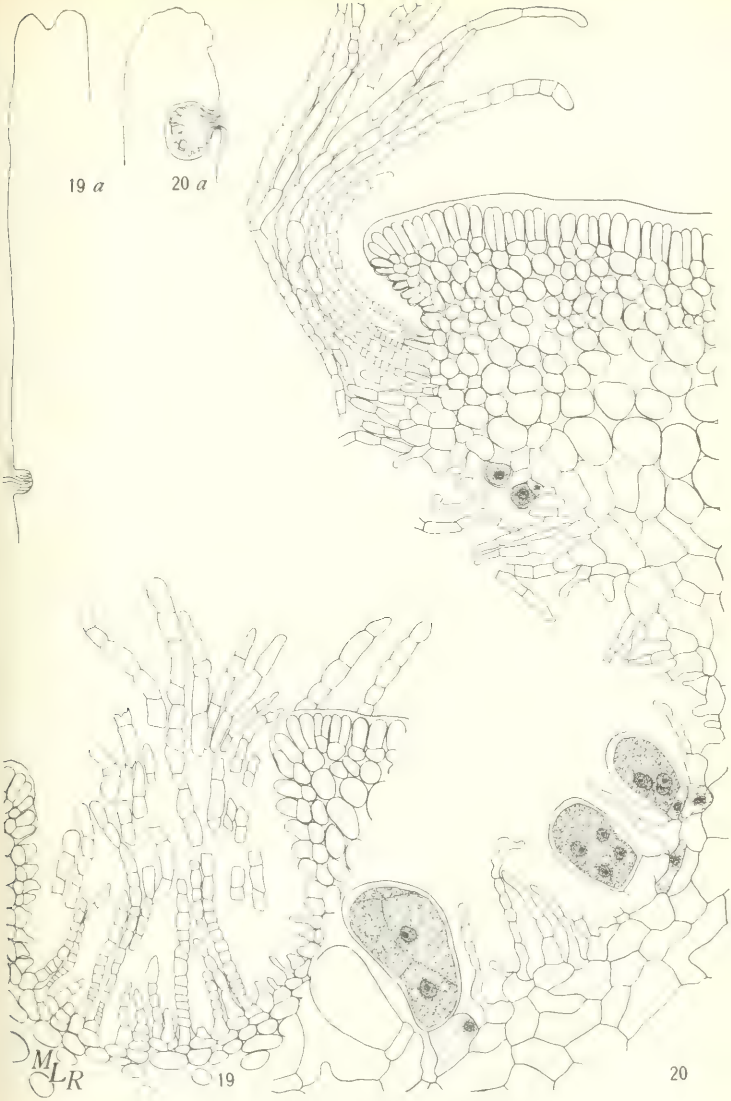

ROE on CONCEPTACLE OF FUCUS

With the author's regards

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DECAY AND SOIL TOXINS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 213

GEORGE B. RIGG

The decomposition products of a specific plant organ and their effects on the growth of other plants furnished the point of attack for the work on toxicity reported in this paper. The material used was the rhizomes of *Nymphaea advena* Ait. and *N. polysepala* Greene. This material was obtained at intervals from July 1912 to October 1915, at various places in the vicinity of Chicago, Illinois, and Seattle, Washington.

Review of literature

RELATED WORK ON TOXICITY

The organic constituents of soils have been under investigation by workers in the United States Bureau of Soils for 10 years. LIVINGSTON (**12, 13**) found toxic substances, probably organic, in an unproductive soil. SCHREINER (**21**) and his co-workers have isolated from soils more than 25 organic compounds differing widely in chemical character. Some of these (for example, dihydroxystearic acid) have proved harmful to growing plants; some (for example, nucleic acid) have been found beneficial; and some have not been shown to have any effect on the growth of plants. BOTTOMLY (**3**) has found that certain aerobic organisms grow well in peat and form from it compounds that are beneficial to the growth of plants. He suggests that very small amounts of accessory organic substances may be necessary for the growth of plants.

Humic acid has been much discussed as a possible factor in plant growth. Not only the effects of this so-called humic acid, but also the constitution and nature of the substance are in doubt. SCHREINER (**21**) regards it as a mixture of substances. WIELER (**29**) takes the view that humic acids in soils are inorganic acids resulting, for example, from the chemical decomposition of salts.

BAUMAN and GULLY (**2**) have suggested that the acidity of bog water is due to the fact that the cell colloids of the disintegrating plant tissues retain chiefly the basic ions of the salts dissolved in the cell contents of the plant tissues before they began to decay, thus freeing the acid ions. SKENE (**24**) has found that various species of *Sphagnum* thrive best in acid solution because mineral solutions, although usually physiologically harmless, may be ecologically harmful.

Work by LIVINGSTON (**14**), DACHNOWSKI (**6, 7**), the writer (**19**), and others (**20**), indicates that the inhibition from sphagnum bogs of plants other than bog xerophytes is not due to acidity, or to low surface tension, or to high osmotic pressure of the soil solution, but is due in part to the presence of toxic substance or substances in the soil solution.

Many workers (**8, 9, 15 18, 25-27**) have found that cultivated crops and plants grown in cultures have a favorable or an unfavorable influence on other plants growing in the same substratum either at the same time or subsequently. Food supply and toxins have been suggested as means through which this influence may be exerted. CZAPEK (**5**) finds that the roots of plants are injured when the surface tension of the bathing solution is lower than 0.66.

SHERFF (**23**) found in Skokie marsh near Chicago that where the rhizomes of *Sagittaria latifolia* had penetrated the decaying rhizomes of *Nymphaea advena*, they themselves had begun to decay.

STERILE CULTURES OF SEED PLANTS

More or less success has been attained by various workers in attempts to grow seed plants under sterile conditions. HARRISON and BARLOW (**11**) tried sterilization by dry heat, moist heat, sulphuric acid, calcium hydrate, formaldehyde, and mercuric chloride, and abandoned all of these means. They succeeded in getting sterile cultures of certain legumes by treating the unopened pods with mercuric chloride, opening them with flamed forceps, and transferring the seeds to a very small quantity of boiling water in sterile test tubes.

WILSON and HARDING (**30**) tried alcohol, formaldehyde, and mercuric chloride as a means of sterilizing alfalfa seeds, but found that when the seeds were sterile, the germination was very low.

Using a modification of HARRISON and BARLOW'S method, they got alfalfa seedlings which grew in sterile cultures for 4 months.

BROWN (4) found that barley seeds take up water from a fairly strong solution of sulphuric acid and remain uninjured. SCHROEDER (22) found silver nitrate to be a good means of sterilizing wheat. He found that hand-picked wheat endured soaking in a 5 per cent solution of this substance for 24 hours without injury, and for 72 hours with but slight injury. Threshed wheat, however, because of the rupture of semipermeable membranes by the machinery, would not stand such prolonged treatment. ARCHICHOWSKY (1) got a large percentage of sterile cultures of seed plants by the use of formaldehyde and other antiseptic agents on peas, pumpkins, and other seeds.

Solutions and preparations

Both of the species of *Nymphaea* used produce branched rhizomes 3-15 cm. thick and sometimes reaching as great a length as 3 m., although they are more commonly 1 m. or less. The older portions of these rhizomes decay. SHERFF (23) found these rhizomes decaying to within a short distance of the growing apex. The writer has found the decay only in older portions of the rhizome. Sound pieces of the rhizome were collected and the following solutions were made up quantitatively, each solution having a volume of 1600 cc. and containing the solutes obtained by the methods described from 1000 gm. of fresh rhizome. An average of 3 tests on the water contents of the fresh rhizome gives 88 per cent of water. The tests were made by cutting 500 gm. of the fresh material into small pieces and drying it at 105° C.

Solution 1A.—This was the liquid resulting from the decay of 1000 gm. of fresh *Nymphaea* rhizome in redistilled water, freed from solid matter by filtering through cheesecloth, and diluted to 1600 cc. with redistilled water. Molds continued to grow on the surface of this solution. It was amber colored.

Solution 1B.—This was the solution remaining after a duplicate of 1A had been extracted by shaking with an equal volume of ether in a separatory funnel. The ether that dissolved in the water solution was removed by heating to 40° C. and subjecting to a suction of 2 cm. of mercury with an aspirator. Molds continued to grow on the surface of this solution. It had a slightly darker color than 1A.

Solution 1C.—The ether used in extracting 1B was allowed to evaporate spontaneously. The solid remaining was reddish-brown, and only partially soluble in water. This solution consists of 1600 cc. of redistilled water, and all the residue from the ether extraction that would dissolve in that quantity of water at 40° C. No molds grew on this solution, and no scum or turbidity or other evidence of bacterial activity appeared.

Preparation 1D.—This was the solid remaining from filtering 1A. This solid was ground with an equal volume of sand.

Solution 2A.—This was the distillate under reduced pressure (2 cm. pressure), at 40° C., of the liquid and solid products of the decay of 1000 gm. of *Nymphaea* rhizome in redistilled water. This was a clear liquid having the appearance of water. No molds grew upon it, and it showed no evidence of bacterial activity.

Solution 2B.—The solid remaining from the distillation of 2A was dried in an oven at 30° C. and then ground in a mortar. It was then black powder. This was extracted in a Soxhlet apparatus with ether. When the ether was allowed to evaporate spontaneously, a sticky, semi-solid, reddish-yellow substance remained. This was only partially soluble in water. This solution represents 1600 cc. of redistilled water, with all of the ether extract that would dissolve in it at 40° C. It had a light reddish-yellow color. No molds grew upon it, and no evidence of bacterial activity appeared.

Solution 2C.—The solid remaining from the extract of 2B was exposed to air until all odor of ether had disappeared. It was then extracted for 2 hours with 1600 cc. of redistilled water. It was perfectly clear. No molds or evidence of bacterial growth appeared.

Preparation 2D.—This represents the solid remaining from the extract of 2C, ground in a mortar with an equal volume of sand to form a soil.

Solution 3A.—One kg. of fresh *Nymphaea* rhizome was cut into pieces, ground in a meat grinder, and the juice pressed out in a fruit press. The solid remaining was extracted with ether, and afterward exposed to the air until the odor of ether disappeared. It was then allowed to decay in redistilled water. The liquid resulting from this decay was strained through cheesecloth and diluted with redistilled water to 1600 cc.

Solution 3B.—The juice squeezed out in preparing solution 4A was extracted by shaking with an equal volume of ether in a separatory funnel, which was then freed from ether by heating to 40° C., and subjecting to suction (2 cm. of mercury) by means of an aspirator for 24 hours. This, when diluted 1600 cc., constituted solution 3B.

Solution 3C.—The ether used in extracting 3A was combined with that used in extracting 3B. This was allowed to evaporate spontaneously and as much of the residue as possible was taken up in 1600 cc. of redistilled water at 40° C.

Preparation 3D.—The solid matter remaining on the cheesecloth in the preparation of 3A was air-dried and ground with an equal volume of sand to form a soil.

Solution 4A.—This was the water extract of the fresh rhizome made under sterile conditions. The rhizome was cut into small pieces and placed in flasks with water. These flasks were stoppered with cotton and sterilized in an autoclave. This solution stood sterile for 11 months before its toxicity was tested.

Solution 5A.—This was the water solution of the ash from the fresh rhizome.

All sand used in the previous preparations and in the following experiments was either no. $2\frac{1}{2}$ quartz or "Ottawa test." In all cases it was washed in 10 per cent HCl, freed from acid by washing in running water, and finally rinsed with redistilled water.

The "Knop's solution" had the following composition: 1 part KNO_3; 1 part K_2HPO_4; 1 part $Mg\,SO_4$; 4 parts $Ca(NO_3)_2$. This was made up to 0.1 per cent.

Where "tap water" is mentioned, the water used was Chicago city water. Where "Cedar River water" is mentioned, the water used was Seattle city water, which is piped from the river near its origin in a snow-fed lake. Where "Lake Washington water" is mentioned, the water is that supplied from Lake Washington to the botany laboratories at the University of Washington.

Experiments and results

In order to determine the relative toxicity of these various solutions to *Tradescantia*, cuttings of the plant were placed in various dilutions of each solution with redistilled water. In this way the percentage of the solution (that is, the number of cc. diluted to 100 with redistilled water) that would allow the formation of roots but inhibit the production of root hairs was determined.

All of the solutions except 5A were acid to both litmus and phenolphthalein. Their acidity was determined by titrating with N/10 NaOH, using phenolphthalein as an indicator.

Table I gives the toxic limits of these solutions to *Tradescantia* (as previously defined) and their acidity, together with the relative rank of each solution as to toxicity and acidity and the ratios of these.

TABLE I

Solution acidity	Toxicity (per cent)	Rank as to acidity	Rank as to toxicity	Ratio of acidity to toxicity
1A N25/1000	7.5	1	1, 2, or 3	0.27
1B N24/1000	7.5	2	1, 2, or 3	0.25
1C N11/1000	7.5	4	1, 2, or 3	0.12
2A N22/1000	10.0	3	4	0.24
2B N6 /1000	50.0	7	7	0.12
3A N9 /1000	12.5	5	5	0.10
3B N7 /1000	15.0	6	6	0.08
3C N5 /1000	75.0	8	8	0.06

All of the solutions mentioned in table I, except 3B, were neutralized to phenolphthalein with N/10 sodium hydrate, and the effect of both the acid and the neutral solution was tried on *Tradescantia* cuttings. Table II shows the results of the dilutions named on *Tradescantia* cuttings.

TABLE II

Solution	Strength (per cent)	Acid	Neutralized
1A	7.5	Root hairs none	Root hairs normal
1B	5.0	“ “ slightly stunted	“ “ “
1C	5.0	“ “ “ “	“ “ normal
2A	7.5	“ “ none	“ “ slightly stunted
2B	60.0	Roots none	Roots 3–10 mm. long
3A	12.5	Root hairs none	Root hairs slightly stunted
3C	10.0	“ “ slightly stunted	“ “ normal

The toxicity of the first 4 of these solutions when undiluted was not perceptibly lowered by neutralization with sodium hydrate.

A 20 per cent solution of 1A was shaken in a large flask 5 times a day for 10 minutes each time, for 3 days in order to aerate it, and then filtered through a filter paper. Tests on *Tradescantia* indicated that its toxicity was not decreased by this treatment.

A 20 per cent solution of solution A in tap water was shaken with animal charcoal and filtered. The filtrate was colorless and odorless. The solution before this treatment had an amber color and a foul odor. Cuttings of *Tradescantia* were placed in this. In all cases they grew well and produced good roots with normal or only slightly stunted root hairs.

The animal charcoal with which this solution was shaken was used as a soil for cultures of alfalfa seed. Controls of animal charcoal shaken with tap water were run. The growth in the controls was twice as great as the growth in the cultures.

The toxicity of preparation 2D to *Tradescantia* cuttings was tested by planting the cuttings in the preparation in small flower pots. This preparation was further diluted also by grinding with half its volume of sand. For convenience this further dilution is referred to in table III as preparation 2Dx. Controls were run in potting soil. All of the pots in a set were placed together in a large glass dish, so that they stood in about one-fourth of an inch of tap water and thus were all watered alike. Table III shows the results at the end of 14 days.

TABLE III

Medium	Roots	Plants
2D . .	None	Dying
2Dx. . . .	Normal	Healthy
Potting soil . .	Normal	Healthy

Preparation 3D was tried in the same way with similar results. Preparation 1D was tried in the same way with corn and did not prove toxic. Preparations 2D and 3D are not at all toxic to alfalfa.

The toxicity of solutions 1A, 1B, 1C, and 2A to unsterilized corn was tested by planting the seeds in 200 cc. of sand watered with 25 cc. of solution. The cultures were in 500 cc. Erlenmeyer flasks, stoppered with cotton. Controls watered with Knop's solution were run. All of the solutions tested proved toxic, but 2A was found to be less toxic to corn under these conditions than the others. The toxicity of the solutions was shown in the injury to the root tips, causing a great decrease in the total length of root produced as compared with the controls, and thus eventually killing the plants.

The effect of the 8 solutions listed in table I was tried on unsterilized alfalfa in sand. They were all found extremely toxic, many of the plants dying as they emerged from the sand, and the best of them attaining only one-fifth of the height attained by controls watered with tap water.

It was found by tests that tomato seeds did not germinate at all in solution 1A in sand, while controls in sand watered with tap water grew vigorously.

An attempt was made to secure sterile cultures of corn in 500 cc. Erlenmeyer flasks in order to determine whether the presence of organisms in the cultures was a factor in the toxicity of these cultures. The sterility of the cultures was tested by making bouillon cultures from various portions of the sand and the seeds at the end of the experiment; 65 per cent of the cultures proved sterile.

Three problems were to be solved in working out a method of securing sterile cultures of seed plants: (1) the sterilization of the flasks and contents; (2) the sterilization of the seeds; (3) the transfer of the sterile seeds to the sterile flasks under sterile conditions.

The cotton-stoppered flasks, containing 200 cc. of sand and 20 cc. of the solution, were sterilized for 1 hour at 15 lbs. pressure in the autoclave. A solution of silver nitrate was used as a means of sterilizing the seeds. It was found by experiment that corn would germinate well after treatment for 1 minute with N 300 $AgNO_3$ and subsequent washing with water. As a means of making the transfer of the seeds to the flask, the box previously used by JENSEN (**11a**) was used. When the lid of the box was closed, the operator could thrust his hands into the gloves and work without danger of contaminating the cultures from any source outside of the box. Since the entire top and part of the front of the box were of glass, all articles inside of the box could be seen readily by the operator.

The entire inside of the box, including the side of the gloves and sleeves exposed to the air of the box, and the outside of all flasks and other articles placed in the box, were treated thoroughly with a 15 per cent solution of glycerine saturated with carbolic acid. All of the cultures prepared were thus more or less exposed to carbolic acid fumes. This solution was applied by means of a sponge attached to a short stick. It was found that flasks and dishes stuck to the paint treated with this mixture, so that a false bottom of glass was placed in the box. The stoppers of the sterilized flasks (including 2 of sterile water) were flamed, and the flasks placed in the box. Four tall stenders with ground glass covers were sterilized in an oven for 5 hours at 120° C., and placed in the box. A bottle of N/300 $AgNo_3$ was also placed in the box, as were also a pair of 10-inch brass forceps and a waste jar containing a little of the glycerine carbolic acid solution. Everything in the box having been treated with the antiseptic solution, the cover of one of the stenders was removed and dry corn placed in the stender and the cover quickly replaced. In selecting corn for this purpose, care had been taken to secure smooth kernels that would offer little opportunity for the lodgment of air bubbles in the dent or elsewhere.

The box now remained closed over night, to allow any organisms in the air to settle to the bottom of the box and be held by the glycerine solution. In the morning the operator thrust his hands into the gloves, poured $AgNO_3$ on the seeds, left it on for 1 minute, poured it off into the waste jar, and then washed the seeds in several changes of sterile water, finally allowing them to soak in it for several hours, and rinsing them again. The tips of the forceps now were washed thoroughly in the sterile water and the transfer of the seeds was made. In some cultures the seeds were thrust down into the sand by means of these forceps, and in some cases they were left on the surface. After 2 or 3 cultures had been made, the forceps were placed in the antiseptic solution for a few minutes and again rinsed in sterile water. After the cultures were prepared, they were removed from the box and placed near a window in the laboratory.

The results showed that solutions 1A, 1B, 1C, and 2A (these were the only ones tried) are just as toxic under these conditions as when organisms were known to be present in abundance. Since 65 per cent of these cultures were shown (by bouillon cultures from them at the close of the experiment) to be sterile, it is evident that the presence of organisms is not a necessary condition of the toxicity of these solutions.

Solutions 1A, 1B, and 1C likewise proved toxic to alfalfa in cultures prepared as previously described for corn. The same precautions for securing sterility were observed, but tests of sterility were not made.

The toxicity of solution 4A to *Tradescantia* cuttings was tested as follows: wide-mouth 50 cc. bottles were filled with dilutions of the following strength: 20, 15, and 10 per cent. Into each of these was placed a cutting of *Tradescantia*. The mouths of the bottles were left open. At the end of 16 days no root hairs had formed on any of these plants and all of the plants were showing signs of death. The controls in Lake Washington water all had abundant root hairs and were in healthy condition.

Other sets of bottles were prepared as previously described, except that the mouths were stoppered with cotton and the bottles containing the liquids were sterilized in the autoclave. The dilutions were as follows: undiluted, 20, 15, and 10 per cent. Each cotton stopper was then displaced just enough to allow a cutting of *Tradescantia* to be placed in the liquid. None of these plants developed normal root hairs. All of them except those in the 10 per cent dilution showed signs of death at the end of 16 days. Turbidity due to the action of organisms was evident in all of these, and molds grew on some of them.

Flasks (500 cc.) were prepared, cotton-stoppered, each containing 180 gm. of sand and 18 cc. of solution. These were autoclaved at 12 lbs. pressure for 1 hour. Solution 4A was used pure and also in the following solutions: 20, 15, and 10 per cent. Controls with tap water were also run. Corn was treated with N/100 silver nitrate for 2 minutes, then with sterile forceps 5 kernels were placed in each flask. At the end of 16 days the growth was noticeably greater in all of the controls than in any of the solutions. All of the flasks except 2 (one control and one 15 per cent) had molds growing in them. Bouillon and also agar cultures were made from each of these, and the 15 per cent flask proved to be sterile. The other was not sterile. These 2 flasks were kept a week longer and the growth in the control was much better than that in the 15 per cent solution.

The transfers of the corn in these cases were made in the laboratory, without the use of the sterile box just described. It is hoped that by autoclaving the flasks at a higher pressure, and treating the seeds with the silver nitrate for a longer period, a larger number of sterile flasks could be obtained.

Solution 1A was filtered and the fresh filtrate was immediately saturated with ammonium sulphate. The sulphate was added gradually and the solution was shaken after each addition. No precipitate appeared at once, but when it had stood over night a considerable amount of a brownish precipitate was present. Some of the precipitate was at the surface of the liquid, some had settled to the bottom, and some particles were in suspension in the liquid.

The precipitate was filtered off and redissolved in a volume of Cedar River water equal to the original volume of the solution. Both filtrate and precipitate were then dialyzed in dialyzing tubing in running water for 11 days. At the end of this time they showed no precipitate with barium chloride. The filtrate and the precipitate were then tested for toxicity by placing *Tradescantia* cuttings in them. Root hairs were formed in both, but their development was poorer in the solution of the precipitate than in the filtrate.

In preparing solution 5A, 2.83 gm. of ash were obtained from 500 gm. of fresh rhizomes. This is 4.7 per cent of the dry weight. Approximately half of this went into solution when shaken with 800 cc. of Cedar River water at 18° C. The solution was basic to litmus. In all cases tried with 5A and its duplicates the toxicity to *Tradescantia* cuttings was so marked that practically no root hairs developed and the plants soon died. When dilutions were tried it was found that all dilutions down to 10 per cent (10 cc. solution to 90 cc. water) inhibited root hair production; in 5 per cent dilution root hairs developed normally.

A solution was prepared from each of the following substances, by allowing a quantity of it to decay in redistilled water: potato, turnip, rhizome of *Castilia odorata*, and rhizome of *Typha latifolia*. These solutions approximated the strength of solution 1A prepared from *Nymphaea*. They all proved toxic to *Tradescantia* cuttings, but to a less degree than 1A did. Their toxicity was in the order named.

Discussion

It is evident from the data given that even very dilute solutions of the products of the decay of *Nymphaea* rhizomes are toxic to *Tradescantia* cuttings in water cultures and the seedlings of tomato, alfalfa, and corn in sand cultures.

Although the products of the decay of the subterranean parts of other plants proved toxic, the toxicity of the products of the decay of *Nymphaea* rhizomes was considerably greater than that of any other plant parts experimented on. While it is possible that toxicity from decay is rather common, *Nymphaea* seems to merit particular attention in this regard. The dilution of solution 1A that entirely inhibited the formation of root hairs on *Tradescantia* cuttings contained in each cc. the products of the decay of 4.7 mg. of fresh rhizome. Since only 12 per cent of the fresh rhizome is solid matter, the amount of solid whose decay contributed to the solutions in each cc. of the toxic solution was 0.56 mg.

The fact that the solutions listed in table I were all acid, and that their toxicity was largely destroyed by neutralization with sodium hydrate, would seem to suggest acidity as a large factor in the toxicity. The toxicity is not proportional to the acidity as determined by the titration method. It may be proportional, however, to the H ion concentration, or some other factor may be effective. The fact that the toxicity of 1A, 1B, 1C, and 2A when undiluted was not reduced by neutralization with sodium hydrate seems to emphasize further the presence of some other factor. It is possible that the osmotic pressure of such a concentrated solution was high enough to cause injury, although it has been shown elsewhere (**20**) that this is not the cause of the toxicity of the very dilute solutions. Antagonistic action on permeability might also be a possible factor in the lowering of toxicity on the addition of sodium hydrate.

The fact that the toxicity of solution 1A was not destroyed by the aeration here reported does not necessarily mean that the toxicity cannot be removed by oxidation.

The removal of the toxicity from solution 1A by shaking it with animal charcoal is probably to be explained as an absorption phenomenon.

The only importance attaching to the toxicity of the soils (preparations 1D, 2D, and 3D) is that not all of the water-soluble toxic materials had been washed out of them by the treatments to which they had been subjected.

The products of the decay of *Nymphaea* rhizomes are toxic, not only to *Sagittaria* and *Tradescantia*, but also to agricultural plants. Apparently the toxicity of solution 1A to tomato, alfalfa, and corn is in the order named.

While the box used for transferring the sterile seeds to sterile flask cultures is fairly efficient, the method is somewhat slow and tedious, and it is believed that fairly good results may be obtained without its use. It seems very desirable to extend our knowledge of the growth of seed plants in the absence of other organisms.

In the one case mentioned, the toxicity of the products of this rhizome to corn seemed to be independent of the presence of organisms, either at the time of dissolving the toxin from the rhizome, or at the time of its action on the growing plants.

The fact that the toxicity of solution 1A, even when undiluted, can be removed by precipitation with ammonium sulphate seems to suggest that the presence of colloidal matter may be a considerable factor in toxicity of that solution.

An alkaloid similar to nupharin is reported by WEHMER (28) in the rhizome of *N. alba*. He also reports fat and organic acids. These also represent possible factors in the toxicity. It seems possible that the toxicity may be due partly to products formed during decay and partly to products merely released by this decay.

The toxicity of the water extract of the ash is possibly accounted for on the basis of the presence of one or more basic substances. WEHMER states that the rhizome of *Nymphaea alba* contains

9.86 per cent of ash with the following composition: CaO, 8.2 per cent; Cl, 15.2 per cent; Na_2O, 48.47 per cent; K_2O, 9.86 per cent; P_2O_5, 14.36 per cent. The presence of sodium hydroxide or of sodium carbonate in the water solution of this ash seems probable.

There seemed to be 5 possibilities as to the cause of the toxicity of the products of the decay of this rhizome: (1) the presence of the organisms; (2) toxicity due to ionization; (3) the presence of toxic molecules; (4) high osmotic pressure of the solutions; and (5) low surface tension of the solutions. Of these, (1) seems to be practically eliminated by the work here reported. Apparently the presence of organisms, either at the time of the formation of the solution or at the time of their action on growing plants, is not a condition necessary for their toxic action. Work elsewhere reported (**20**) disposes of (4) and (5). This leaves ionization and toxic molecules as probable causes of the toxicity. The relative importance of these two is not fully determined by the work here reported. It is evident, however, that the entire toxicity cannot be ascribed to one substance. If we should suppose that the toxicity of all of the solutions obtained from this rhizome was due to only one substance, it would have to have the following properties: (1) soluble in water; (2) soluble in ether; (3) volatile at 40° C., 20 mm. pressure; (4) stable at 250° C., 15 lbs. pressure; (5) absorbed by animal charcoal; and (6) precipitation by ammonium sulphate.

It seems probable that there are at least 3 classes of substances here that are somewhat toxic: (1) colloids, (2) very volatile substances, and (3) certain bases.

Summary

1. The products of the decay of *Nymphaea* rhizomes are toxic to *Tradescantia* cuttings, and to tomato, alfalfa, and corn, even in very dilute solutions.

2. All of the solutions prepared except the one from the ash were acid, but the amount of acidity was not proportional to the degree of toxicity.

3. The toxicity of the very dilute solutions (but not of the strong solutions) was nearly destroyed by neutralization with sodium hydrate.

4. The toxicity of solutions resulting directly from the decay of the rhizome is destroyed by shaking them with animal charcoal.

5. The water extract of the fresh rhizome made under 15 lb. pressure in an autoclave is toxic to growing plants.

6. The rhizome and the products of its decay contain some ether-soluble toxic material.

7. Some of the toxic material in the products of the decay of these rhizomes is volatile at 40° C.

8. The toxicity of even concentrated solutions resulting directly from the decay of these rhizomes can be removed by precipitation with ammonium sulphate.

9. The water extract of the ash from these rhizomes is basic and toxic.

10. The products of the decay of potatoes, of turnips, and of the rhizomes of *Castalia odorata* and *Typha latifolia* are also toxic to *Tradescantia*, but slightly less so than those of *Nymphaea*.

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STANGERIA PARADOXA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 214

(WITH PLATES XXIV-XXVI AND ONE FIGURE)

CHARLES J. CHAMBERLAIN

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STANGERIA PARADOXA

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(WITH PLATES XXIV–XXVI AND ONE FIGURE)

Stangeria is the most fernlike of all the cycads; in fact it was described originally as a species of *Lomaria*, one of the Polypodiaceae, and the mistake was not corrected until the cones were discovered. The genus may be monotypic, with *Stangeria paradoxa* as its single polymorphic species. That its general habit, as it appears in the field, is extremely variable is beyond question; and that under cultivation in conservatories and botanical gardens it becomes quite different from the wild form is also apparent.

A plant called *Stangeria eriopus* in the New York Botanical Garden has produced fine crowns of leaves, surpassing anything one is likely to find in the field, and it has had at least one ovulate cone (**10**). A specimen of *S. paradoxa* in the Botanic Garden at Sydney, Australia, had in 1911 a fine display of leaves and 8 staminate cones; while another plant in the same garden had 5 ovulate cones and an equally fine display of leaves. In the Sydney plants the leaves were not in a single crown, but on subterranean branches with only a few leaves on a branch. In both the Australian and the African gardens *Stangeria* produces leaves and cones more freely than in the field, and both cones and leaves are larger than in field specimens. The cultivated specimens are more beautiful and luxuriant, and differ so much from plants in the field, that some observers might make new species of them. In the Botanical Garden at Durban, South Africa, Mr. WYLIE showed me a vigorous *Stangeria* with leaves a meter long. The leaflets were 45 cm. long, and so deeply incised that they were almost pinnate. Some taxonomists, doubtless, would regard this plant as a distinct species. In *Dioon* and *Bowenia* the character of the leaf margin is so rigidly fixed and so persistently transmitted from one generation to another that it is entitled to rank as a specific character; but in *Stangeria* the character of the margin is so fluctuating that even the almost bipinnate Durban plant should not be regarded as specifically different from forms with entire leaflets.

Field observations

My field observations on *Stangeria* began at Ngoye, near Mtunzini in Zululand, about 100 miles north of Durban, and extended to East London, about 275 miles south of Durban. *Stangeria* certainly extends farther north, just how much farther I was not able to determine; and its western limit is west of East London. I did not find it at Port Elizabeth, about 150 miles west of East London, or even at Grahamstown, or Trapps Valley, about half-way between East London and Port Elizabeth; but Mr. GEORGE RATTRAY, of Selborn College, East London, has reported it from Port Elizabeth, and he regards this as about the western limit of its range. His extensive knowledge of South African cycads in the field enables him to speak with authority upon their geographical distribution.

In the field, *Stangeria* presents two forms, one growing on the open grass veldt and the other in the shade of bushes or trees, the shaded form being much larger and resembling more nearly the cultivated specimens. PEARSON (**9**) has contrasted the two forms as *Stangeria paradoxa*, the species originally described from the shaded Natal form, and *Stangeria* sp., the open grass veldt form; but he had seen both forms in the field, and consequently hesitated to give a specific name to the second form. RATTRAY, who lives in a *Stangeria* region, and who has studied the genus throughout its range, believes there is only one species. Before I left South Africa, I had come to the same conclusion. I dug up several plants from the East London grass veldt and sent them to our greenhouse at the University of Chicago, where after three years of the usual unnatural conditions they are producing leaves and cones as large as those of the bush veldt form.

Near Mtunzini in Zululand the grass veldt stretches for miles, rolling and hilly, broken by huge rocks of granite and gneiss, and occasionally with exposed surfaces covered only with *Selaginella* and lichens. The grass is 30–40 cm. high, and appearing above it are the leaves of *Stangeria* (fig. 1). The leaves are larger and more numerous than in the East London district, three or four leaves being common, and some stems having fine crowns of five or six leaves.

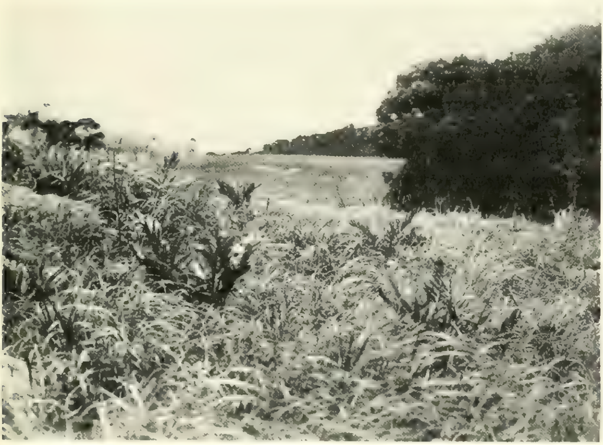

FIG. 1.—*Stangeria paradoxa* growing in the grass veldt at Ngoye, near Mtunzini, Zululand.

The plants are fairly abundant, as many as 20 being in sight at one time; but the specimens are scattered, with no crowded masses like the thickets of *Macrozamia spiralis* and *Bowenia serrulata* in Australia. Although adult plants are numerous, cones are rare. At the time of my visit, the middle of January, 1912, the staminate cones had rotted or dried up, and the ovulate cones were falling to pieces. The few seeds which were secured showed embryos in early suspensor stages.

In the Mtunzini bush, which is particularly dense and rich in ferns, only a few plants of *Stangeria* were seen, but they were vigorous, with three or four leaves about twice the size of those in the open grass veldt. Not a single cone or seed was found in the bush. It is said that baboons are very fond of the seeds and carry them away as soon as the cones reach their full size.

Associated with *Stangeria* in the Ngoye grass veldt is *Encephalartos brachyphyllus*, a species with small cones and tuberous, subterranean stems. I saw one tall specimen of *E. Altensteinii bispinosus*, which is called also *E. Woodii*. It is said that there is not another plant of this species within 50 miles. I did not see a single specimen of any species of *Encephalartos* in the Ngoye bush veldt.

On the grass veldt at East London, *Stangeria* is not nearly so large or so abundant as in Zululand, but its appearance is the same, the leaves projecting a little above the grass, so that most of the plants within a distance of 100 m. can be seen. In this region *Stangeria* is associated with *Encephalartos Altensteinii* and *E. villosus*, while *E. cycadifolius* grows within a few miles.

The stem of *Stangeria* is tuberous and entirely subterranean, with a strong main root and weak branches. It grows in soil so hard and stony that both care and labor must be given to secure an uninjured specimen. The stem is quite smooth, the whole leaf breaking off so cleanly that there is never any armor of leaf bases.

Nearly all the plants dug up in the East London region showed more or less branching, a feature already noted by PEARSON and by RATTRAY. Occasionally the stem is simple, but usually it bears 1-4 branches arising from the lower part of the stem, rarely from the upper part. Often the stem and branches are so nearly alike that it is difficult to distinguish which is the main stem and which is the branch. Sometimes the branching condition is betrayed by the leaves, which may be too scattered to belong to a single crown and too crowded to belong to different plants; but it may happen that only a single branch will bear a leaf, and in that case the branching is not discovered until the soil is removed.

The stem is monoxylic, with a single very narrow zone of wood between the large pith and cortex. A stem 9 cm. in diameter had a zone of xylem only 2 mm. wide, and even in this zone the bundles were separated by a large amount of parenchyma. That this plant was an old one was evident, not only from its size, but also from the fact that it had borne several cones. In the field, cones are not abundant, about one coning plant in six being a liberal estimate. No observations have been made to show at what age *Stangeria* begins to bear cones, but some 5-year old seedlings in our greenhouse are only 2 cm. in diameter. We did not see any cones on plants less than 5 6 cm. in diameter, and we should judge that the specimen must have been at least 30 years old, perhaps much older. The display of woody tissue is the scantiest I have ever seen in any cycad of even approximately equal age.

Cone domes are numerous and are of the type already noted for *Dioon*, *Zamia*, and *Ceratozamia* (**13**). The main stem, therefore, is a sympodium, as are any branches which have borne more than one cone.

Sporangia

Although cones are rather infrequent, material can generally be secured in localities where the plants are abundant.

MEGASPORANGIUM.—The ovulate cone looks like that of *Dioon spinulosum*, only very much smaller, the similarity being due to the densely hairy surface and the thin rounded border of the blade portion of the sporophyll. The ovules, during earlier stages of development, are almost completely inclosed by outgrowths of the sporophyll, suggesting the angiosperm condition; but later the ovules grow rapidly and soon protrude beyond the outgrowths, which do not develop much after the ovules reach a length of 4-5 mm. At maturity the ovules have a rich orange color which is quite characteristic.

Early stages in the development of the ovule have been studied by LANG (**5**), and its vascular anatomy has been described in detail by WORSDELL (**4**) and by MATTE (**7**).

Microsporangium.—The staminate cones are long and slender and very symmetrical. They vary greatly in size, those growing on the dry grass veldt sometimes being as short as 8-9cm. in length, while those in the shaded bush veldt may reach a length of 20cm., exclusive of the stalk, which may be more than 5cm. in length. The sporophylls are small and the number of sporangia averages only about 150, which is lower than in any cycad except *Bowenia* and *Zamia*.

We have already stated that *Stangeria* was originally described as a fern, because the leaves so strikingly resemble those of *Lomaria*. The microsporangia are equally fernlike and bear a particularly close resemblance to those of *Angiopteris*. The wall of the microsporangium consists of an outer layer of very thick-walled cells, a very scanty tapetum, and 3–5 intervening wall layers. The thickening of the outer cells is most pronounced at the base, diminishes gradually on the sides, and at the top becomes so thin that it easily breaks, forming a pore through which the dense cell contents escape shortly before the dehiscence of the sporangium.

The earlier stages in the development of the microsporangium and in the origin and development of sporogenous tissue have been so thoroughly described and illustrated by Lang (**2**) that we have not felt it necessary to deal with this phase of the subject; but on account of the peculiar or at least newly described behavior of the chromatin at fertilization, the reduction divisions in the pollen mother cells would have been studied if material in this stage had been available.

Gametophytes

Male gametophyte.—The microspore is formed before any of the wall cells of the microsporangium begin to break down, but before the nucleus of the microspore divides the wall cells begin to disorganize and the tapetum becomes almost indistinguishable. During the two mitoses which result in the formation of prothallial cell, generative cell, and tube cell, the tapetum and the wall cells, with the exception of the hard outer layer, break down and form a dry, homogeneous membrane about the pollen grains, holding them together in one spherical mass. At the time of dehiscence, the grains are dry and powdery, but are still confined by the thin membrane, which often holds them together even after they fall out from the sporangium. Most of the pollen, however, is in condition for wind pollination. Staminate cones of cycads are very frequently infested by insects, both before and after the pollen has been shed, but I have never seen anything to indicate that pollination was being affected by their agency. Besides, the ovules exude a large pollination drop, as in the case of gymnosperms known to be wind-pollinated.

When I reached Zululand, about the middle of January, 1912, the pollen tubes had already discharged; in fact, embryos were beginning to show suspensors. Although the season is somewhat later farther south, I did not find any cones in which the pollen tubes had not discharged. Accordingly, I am under great obligation to other people for material of pollen tube structures.

On January 6, 1910, Professor W. C. WORSDELL, whose extensive researches have so materially advanced our knowledge of the anatomy of cycads, visited Zululand and fixed for me some material showing pollen tubes with the sperms already formed. Mr. W. T. SAXTON, formerly of the South African College, but now of the Institute of Science at Ahmedabad, India, made several collections in the Transkei, and arranged with Miss SARAH VAN ROOYEN, of Kentani in the Transkei, for a close series of stages. The carefully prepared material furnished by Miss VAN ROOYEN yielded an excellent series of stages from the body cell, through the development of the sperm, and up to early suspensor stages in the embryo. The collections also included ripe seeds with mature embryos. Seedlings have been grown both from Professor WORSDELL'S and from Miss VAN ROOYEN'S collections.

The date of pollination was not determined definitely, but material collected in the Transkei on July 17, 1907, showed uninucleate pollen grains with exine and intine well developed. This would indicate that pollen is shed late in July. Plants from that region, now growing in our greenhouse, shed the pollen in January 1916. The sporophylls of an ovulate cone loosened at the same time, just as they do normally at the pollination period. This wide difference is due doubtless to greenhouse conditions and not to the transfer from one hemisphere to another, for *Stangeria* in the Botanic Garden at Sydney was shedding in December 1911. The pollen at the time of shedding shows a prothallial cell, a generative cell, and a tube cell, a condition which I have now observed in all the 9 genera of the family.

Early stages in the development of the pollen tube were not observed, but later stages indicate that they do not differ essentially from the course already described in detail for *Dioon edule* (11). A characteristic view of the pollen tube structures after the pollen chamber has extended entirely through the nucellus is shown in fig. 2. In the tube at the right the body cell has divided, but in the rest the division has not yet taken place. The tube nucleus is regularly near the body cell. The haustorial end of the tube extends in a straight line a few layers of cells below the epidermis, scarcely ever showing any branching, and there are no basal haustoria like those of *Ceratozamia*. While the pollen tube structures of the cycads present many similarities, the differences are probably sufficient for a determination of the genera.

By comparing figs. 2 and 3, which are drawn to the same scale, it is evident that in the later development both the tubes and the sperms increase greatly in size. It is during this rapid increase in size that the spiral band with its immense number of cilia develops from the blepharoplast. The cycad sperm is the largest and most complicated motile cell in either plants or animals. While the series of stages in *Stangeria* is quite satisfactory, the series in *Ceratozamia* is even more complete, thanks to Mr. ALEXANDER M. GAW, of Jalapa, Mexico, who for 10 years has been sending material to supplement my own collections. Besides, the blepharoplast of *Ceratozamia* is larger. Consequently, a more detailed study of the development of the sperm will be reserved for this genus; but some of the more obvious features, as they appear in *Stangeria*, will be presented here.

While the body cell remains elongated during the early stages in the development of the tube, the two blepharoplasts occupy the fore and aft positions; but as the basal end of the tube enlarges and the body cell becomes spherical, the blepharoplasts rotate 90°, so that their axis becomes transverse to the long axis of the tube.

At first, the blepharoplast is a solid, homogeneous body, but as it increases in diameter it becomes vacuolate and soon appears to be little more than a shell containing droplets of liquid (fig. 4). This stage is reached before the body cell divides. During the division of the body cell, the vacuolate blepharoplast breaks up into hundreds of small granules which, for a short time, occupy the space previously occupied by the blepharoplast (fig. 5). The daughter nuclei immediately after the division of the body cell are smaller than the blepharoplasts; but the nuclei increase rapidly in size, while the group of granules remains stationary until it begins to form the ciliated band. The beginning of the band takes place during a rapid increase in the size of the sperm (fig. 6). Many of the granules become elongated and have a distinct tendency to arrange themselves in rows, forming a band one layer of granules in thickness (fig. 7). From the entire surface of one side of this band, stiff bristle-like cilia grow out. The young band is variously twisted and curved, so that the bristles point in every direction, some of them even toward the nucleus, but the band soon turns so that all the bristles face outward (fig. 8). As the band nears the surface, the bristles prick through the cytoplasm and the growth of the cytoplasm at this point is checked, so that a spiral groove results. After piercing the cytoplasm, the bristles grow out into long, slender cilia, which at first are compressed by the wall of the sperm mother cell, but later extend outward and vibrate vigorously when this wall breaks down. The mature sperm varies from 150 to 190 μ in diameter, and its dense nucleus constitutes most of its mass, the diameter of the nucleus being usually only about 20 μ less than that of the entire sperm, so that the cytoplasm containing the spiral band is only a thin sheath. The number of turns in the spiral varies from 5 to 7, but one peculiar sperm was found with a long, narrow apex and 10 turns of the band (fig. 10).

FEMALE GAMETOPHYTE.—In the young nucellus LANG (5) found an axial row of 3 cells, the lowest of which became the functioning megaspore. In my own material the earliest stages showed archegonia shortly before the division which gives rise to the ventral canal nucleus and the egg. The figure for this division was observed only once, but it was not difficult to determine the number of chromosomes as 12. Several preparations show that no wall is formed between the two nuclei, and that the ventral canal nucleus soon disorganizes, while the egg nucleus increases rapidly in size as it moves toward the center of the egg.

Some irregularities were noted. In two cases there were 4 neck cells instead of 2, in one of the two cases the two original cells having divided longitudinally, and in the other transversely. In several cases the central cell of the archegonium had not continued its development and was no larger than the tissue cells of the gametophyte, but the two neck cells were as large and turgid as those of a normal archegonium.

Fertilization

The archegonial chamber at the time of fertilization is moist, but no droplets of fluid can be seen, even with a 16 mm. objective. On the other hand, the pollen tubes are turgid, and as they discharge furnish enough fluid for a limited movement of the sperms. After the nucellus has been removed from the ovule, it is easy to see the sperms swimming in the pollen tube, but practically impossible to observe them, under natural conditions, in the archegonial chamber. The entrance of the sperm into the egg is probably effected as already suggested for *Dioon edule* (**12**). The liquid discharged from the pollen tube is of high osmotic pressure, and consequently draws some liquid from the turgid neck cells, and the lowered turgidity of these cells allows a portion of the upper part of the egg to escape, thus forming a vacuole which draws the sperm into the egg. The careful experiments of MIYAKE (**8**) have shown that the cycad sperm does not respond to chemotactic stimuli, being indifferent even to the material of the egg.

It is quite common for more than one sperm to pass through the neck of the archegonium, but rare for more than one to enter the egg itself (figs. 10, 11, 13). In one case 7 extra sperms had passed through the neck; in another case 4; in several cases 3; and in many cases 2 or 1. The apex of the extra sperm is usually directed toward the main body of the egg, and in such cases the sperm becomes more or less imbedded, doubtless on account of the movements of the cilia; but when the axis is not directed in this way, the sperm is not likely to cause so deep a depression (figs. 10, 11, 13). The ciliated band is so conspicuous and persists so long that a complete series of sections will show, even to the close of the free nuclear period, whether more than one sperm has entered the egg. The photomicrograph (fig. 24) shows a part of the spiral band of the sperm whose nucleus has fused with that of the egg; and also shows two more sperms which have passed through the neck, one of which has become almost completely imbedded in the egg.

The behavior of the chromatin during fertilization has not yet been described for any cycad, but WEBBER (**6**) figured and described the sperm nucleus of *Zamia* imbedded in the egg nucleus, and IKENO (**3**) found the same condition in *Cycas*. In *Stangeria* the behavior is the same (fig. 9). Both nuclei, at this stage, are in the resting condition. Whether they continue in this condition as fusion proceeds, or break up into two groups of chromosomes, as in *Pinus* and several other conifers, has not been observed as yet. However, the structure of the first mitosis of the fertilized egg indicates that the resting condition is soon followed by the formation of chromosomes by each of the fusing nuclei. The number of chromosomes, as counted at the equatorial plate stage of the first mitosis, is 12; but later free nuclear divisions and also mitoses in root tips show that 24 is the diploid number; further, the mitosis at the formation of the ventral canal nucleus and egg shows 12 chromosomes, proving that this is the haploid number. With the conspicuous spiral band indicating the entrance of a sperm, parthenogenesis is out of the question. HUTCHINSON'S (**14**) recent study of fertilization in *Abies*, from an abundance of material in critical stages, which it has been my privilege to examine, furnishes an explanation of this apparently haploid, but really diploid condition. HUTCHINSON (**14**) finds that the spirems of the two conjugating nuclei segment into chromosomes, as has been described for various conifers; but, after the segmentation, the chromosomes unite in pairs, just as in the prophase of the heterotypic mitosis. Each pair then segments transversely and the two longitudinal parts separate, so that 4 chromosomes are formed from each pair and the diploid number is established. In *Stangeria*, 4 eggs were found which showed the first mitosis, all of them in the early equatorial plate stage. In all of these the number of chromosomes is 12, but the double character of the chromosomes is evident; and since 12 and 24 are the haploid and diploid numbers, I believe that there is a pairing of chromosomes at fertilization, as described by HUTCHINSON (**14**) for *Abies*.

Embryogeny

FIRST MITOSIS.—At the first division in the fertilized egg, the mitotic figure is surrounded by a remarkable display of achromatic structures, arranged in irregular nets or grouped into cones resembling half-spindles (figs. 10, 11, 25). This achromatic area easily identifies the first mitosis, even when a second sperm has entered and divided, since the achromatic area surrounding the dividing sperm is much smaller (fig. 11). The division of an extra sperm was observed in two cases, and in both the mitotic figure showed 12 chromosomes. After the first mitosis the achromatic display gradually diminishes, but is still quite conspicuous as late as the fourth or fifth division. After the first mitosis, however, the display consists almost entirely of slender threads like the spindle fibers (fig. 26).

FIRST FREE NUCLEAR PERIOD.—According to previous accounts of the embryogeny of cycads and *Ginkgo*, there is a regular series of free nuclear divisions, so that the nuclei at the successive divisions number 2, 4, 8, 16, 32, 64, 128, 256, 512, and in some cases 1024, with only such variations as might be expected from the occasional failure of a nucleus to divide. In *Dioon edule* (**12**) it was noted that the 8th, 9th, and 10th mitoses were irregular, especially in the upper part of the proembryo, so that the number of nuclei beyond the 256-nucleate stage was likely to vary widely from the theoretical estimate.

In *Stangeria*, irregularities begin to appear earlier and are more pronounced. The first 4 mitoses, giving rise to 2, 4, 8, and 16 nuclei, are likely to be strictly simultaneous and regular, and the 32-nucleate stage was observed in two cases; but after the 16-nucleate stage (fig. 12), the number of nuclei not only varies from the anticipated 32, 64, 128, etc., but the discrepancies are great. An examination of a large number of cases showed that the free nuclei in the upper half of the proembryo were often smaller and more numerous than those in the lower half. The explanation was soon found, for the nuclei in the upper half may divide simultaneously, while those in the lower half remain in the resting condition (fig. 14). This would double the number of nuclei in the upper part, and naturally the nuclei would be smaller. A counting of nuclei in several complete series of sections indicated that such a division had taken place. When the lower nuclei divide, the upper ones divide also, even if they are already much more numerous (fig. 13). There is one important exception to this statement which will be considered later.

While such nuclear conditions are frequent, they are not universal, for two series showed approximately 512 nuclei of uniform size and uniformly distributed throughout the egg, thus indicating that there had been 9 simultaneous divisions. With the 9th or sometimes the 10th nuclear division the free nuclear period comes to a close.

At any time after the 7th division there may be an evanescent segmentation of the protoplasm, especially in the upper part of the proembryo (fig. 15). We have already described such a condition in *Dioon edule* (**12**), and while we have not yet published the details, we may state that in *Macrozamia*, *Encephalartos*, and *Cycas* the segmentation is much more complete than in *Stangeria* or *Dioon*, and is more permanent, since cellulose walls are formed.

As already stated, the haploid and diploid numbers of chromosomes are 12 and 24. The number 24 is fairly stable in the lower part of the proembryo; but in the upper part there are frequent and extreme variations, the numbers ranging from the theoretical 24 down to a single chromosome. If many of the figures should show just 12 chromosomes, the number could be explained by the continued division of an extra sperm, the first division of which has already been noted; but no such cases were observed. The cause of the irregularity was not determined.

POLARITY.—A striking feature of the free nuclear period is the frequency of a definite polarity. During the earlier divisions, the nuclei may be fairly evenly distributed, but still in two recognizable groups (fig. 12). Very often, however, the grouping is conspicuous, with a considerable space separating the upper and lower groups (fig. 13). Sometimes the middle third of the proembryo is entirely free from nuclei; sometimes the nuclei are distributed throughout, but are smaller and more numerous above; sometimes the nuclei are dividing above but resting below, and later they divide below while the nuclei above are resting; there may be evanescent segmentation above, but no trace of it below; later, there is a migration of nuclei toward the base, followed by segmentation below, with none above; the embryo is formed below, while the upper region furnishes nutrition. In fact, there is a constant manifestation of polarity from the appearance of the archegonium initial throughout the life of the plant. The source of nutrition is probably the cause of a part of these phenomena, but much is still to be explained.

CAUSE OF FREE NUCLEAR PERIOD.—Why the cycad embryogeny should begin with a period of free nuclear division is not hard to imagine. It would seem a physical impossibility for the small mitotic figure of the first division to segment the comparatively immense mass of the egg. Further, the large amount of nutritive material doubtless causes the nuclear divisions to follow in rapid succession. That they do follow in rapid succession is shown by the fact that the 2-, 4-, and 8-nucleate stages are very rarely found; and also by the fact that nuclei which have reached the resting condition are not likely to be found before the 16-nucleate stage. Further, a glance at the illustrations accompanying this paper will show that the mitotic figures, nearly 100, are all in the equatorial plate stage, a fact which indicates that other phases are comparatively rapid.

The small size of the mitotic figure, the great mass of the egg, and the rapid succession of mitoses doubtless cause the free nuclear period which characterizes not only cycads, but nearly all living gymnosperms. As the divisions proceed and the mass of the nucleus approaches that of the surrounding cytoplasm, segmentation, which may be more or less evanescent, appears; and later permanent segmentation with the formation of cellulose walls takes place. In the Bennettitales and earlier cycadophytes the seeds were much smaller than in any of the living cycads yet studied. It would be interesting to know the embryogeny of these forms.

An examination of the tiny *Zamia pygmaea* might show a comparatively short free nuclear period, but I have not yet been able to secure it. *Ginkgo*, with its comparatively small eggs, has only 7 free nuclear divisions, the walls appearing with the 8th division. *Selaginella* and *Isoetes* have a short period of free nuclear division at the germination of the megaspore, but none of the living heterosporous pteridophytes has any free nuclear division in the embryogeny. These stages have not yet been described in any fossil heterosporous pteridophyte, but some of the paleozoic forms had megaspores much larger than those of *Isoetes* or *Selaginella*. Doubtless, with the increase in the size of the egg, a free nuclear period became established, becoming more and more extensive as the egg became larger, until it reached its maximum in some of the living cycads, which have eggs 5 or 6 mm. in length, and which may have more than 1000 free nuclei before wall formation begins. Among the coniferophytes, *Ginkgo* has the most extended free nuclear period yet known. While we believe that the *Ginkgo* condition represents a culmination, it is not so clear that genera with less and less extended free nuclear periods in the embryogeny represent a reduction series, ending in forms like *Sequoia*, in which a cell wall follows the first mitosis in the fertilized egg. This may seem rather speculative, but we believe enough has been observed to warrant our theory that the origin and development of the free nuclear period has been due to a progressive increase in the size of the egg.

MIGRATION OF NUCLEI.—As soon as the first free nuclear period has come to a close, many of the nuclei in the upper and middle portions of the proembryo migrate to the bottom (figs. 16, 17, 18, 27). That nuclei actually move to the basal region is shown by the structure of the cytoplasm and arrangement of the nuclei, and also by the relative numbers of nuclei in the upper and lower parts of the proembryo. The cytoplasm becomes vacuolated in the lower portion, before any movement of the nuclei begins (fig. 15); but during the movement and in succeeding stages the vacuolated condition extends throughout. As the nuclei move to the base of the proembryo, the cytoplasm at that point becomes denser and denser, until it contrasts rather sharply with the vacuolated structure above (fig. 18).

SECOND FREE NUCLEAR PERIOD.—After the migration of nuclei, there is a second period of simultaneous free nuclear division at the base of the proembryo, but the nuclei above do not divide (fig. 19). The mitotic figures of this division were observed in only two cases, but in two others there was evidence that such a division had recently occurred. Since so few cases were observed, the extent of this free nuclear period could not be determined, but early stages in the cellular period would indicate that there are probably only two or three simultaneous mitoses before wall formation begins. The cause of this second period is probably the dense basal accumulation of cytoplasm during the migration of nuclei. The embryo, the suspensor, and some cells which remain within the limits of the egg are all formed from cells resulting from the second free nuclear period, the nuclei and cytoplasm above being resorbed by the growing embryo.

IKENO (**3**), in his classic account of *Cycas revoluta*, described free nuclear mitoses at the base of the proembryo, with amitotic divisions above, but did not give any further description of the embryogeny. His investigation dealt chiefly with the pollen tube structures and oogenesis. TREUB (**1**), who had previously made a study of *Cycas circinalis*, did not mention such a stage.

FORMATION OF WALLS. -At the close of the second free nuclear period, with the last simultaneous mitosis, there is a simultaneous formation of cell walls (fig. 20). At the middle of the cellular region there are about three layers of cells with cellulose walls, and at the edges only one layer. Above these cells with cellulose walls there is a segmentation of the cytoplasm, but the walls do not reach the cellulose stage and are weak and evanescent. Within the cellular region, mitoses are no longer simultaneous, and each nuclear division is followed by the formation of a wall. The region soon becomes sharply marked off from the cytoplasm above (fig. 21). Later, the peripheral walls of the cells bordering upon the cytoplasm become thickened, so that the cellular region is still more sharply defined (fig. 23). The thickening is mucilaginous. The free nuclei in the cytoplasm may undergo occasional divisions, giving rise to numerous small nuclei (fig. 21), while the cytoplasm bordering upon the cellular region becomes denser, losing more or less completely its vacuolated appearance as it is resorbed by the rapidly developing embryo. Before the appearance of cotyledons, the young embryo has resorbed all the cytoplasm and nuclei of the free nuclear region, which becomes an empty shell, maintaining its contour only by the rigidity of the tough egg membrane. All the structures of this region, however, are finally crushed by the backward thrust of the suspensor, which is stopped only by the stony coat of the seed.

DIFFERENTIATION OF CELLULAR REGION.—Differentiation of the cellular region begins almost as soon as it is marked off from the free nuclear region above it. At first there is seen a differentiation into two general regions: the upper, in contact with the free nuclear region, consisting of larger cells; while the cells of the lower are much smaller, but denser and more numerous (fig. 21). Some of the upper cells, especially in the center, increase greatly in size and become actively haustorial; it is through these that the contents of the free nuclear region pass down to the developing embryo (fig. 23).

The smaller, denser cells then become differentiated into two regions, the lower consisting of a single layer of large cells which looks like a dermatogen, and above it several layers of small, dense cells (fig. 22). Most of the outer cells become haustorial, especially at the center. These cells partly digest and partly crush the egg membrane, and the young embryo begins to advance into the endosperm (fig. 23). Only a small portion of the egg membrane is ruptured, and the advancing embryo is extremely narrow in comparison with those of cycads previously described. The region of small, dense cells contributes most of the suspensor and all of the periblem and plerome of the embryo.

Stages in the development of the body regions of the embryo are still lacking in my material, but will doubtless be found in collections now being made in South Africa. From the mature embryo up to seedlings with several leaves the series is fairly complete, and some account will be given at another time in connection with a study of seedlings of other genera.

This paper, like several earlier papers on cycads, is largely descriptive. Since material is in hand for a fairly complete study of all the genera, it seems wiser to reserve theoretical considerations until the available evidence has been examined.

Summary

1. *Stangeria* is probably monotypic, with *S. paradoxa* as its single polymorphic species.

2. At fertilization there is a pairing of chromosomes resembling the pairing in the heterotypic mitosis, so that the number during the metaphase of the first division is apparently haploid, although really diploid.

3. There are two free nuclear periods in the embryogeny, the first comprising 9 or 10 simultaneous mitoses and extending throughout the proembryo, and the second with only 2 or 3 mitoses and confined to the lower part of the proembryo. The embryo and suspensor are formed from the second series.

4. There is an evanescent segmentation of the entire egg, as in *Dioon*.

5. The young embryo is very narrow and its haustorial structures are more conspicuous than in any other cycad yet described.

6. Polarity, which may appear even at the beginning of embryogeny, becomes more and more marked as development proceeds.

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EXPLANATION OF PLATES XXIV–XXVI

Most of the sections were cut at 5 μ; none was thicker than 10 μ. With the exception of figs. 4–8, the drawings were reconstructed from two or more sections. Figs. 12 and 13 are reconstructed to show all the nuclei of these proembryos.

FIG. 1.—Text cut, showing *Stangeria* in the field.

FIG. 2.—Nucellus with pollen tubes showing prothallial cell, stalk cell, body cell with blepharoplasts, and tube nucleus; in one tube (at the right) the body cell has divided; ×50.

FIG. 3.—Later stage: mature sperms are about to be shed; ×50.

FIG. 4.—Blepharoplast, much vacuolated, shortly before breaking up into granules; the body cell has not yet divided; ×365.

FIG. 5.—The blepharoplast has broken up into an immense number of small granules; the nucleus of the young sperm is smaller than the blepharoplast of the preceding figure; the median portion of the spindle can still be seen; ×365.

FIG. 6.—The granules have increased in size and number and are becoming arranged into a flat band; note the great increase in the size of the nucleus; ×365.

FIG. 7.—Transverse section of a band at the stage shown in fig. 6; ×365.

FIG. 8.—The bristle-like cilia beginning to develop from the band; some are directed toward the nucleus; ×365.

FIG. 9.—Fertilization: the sperm nucleus entering the egg nucleus; the ciliated band remains at the top of the egg; ×42.

FIG. 10.—First mitosis in the fertilized egg: at the top, 3 sperms which have passed through the neck, but have not entered the egg; the ciliated band of the sperm which effected fertilization is easily seen; ×42.

FIG. 11.—First division of fertilized egg: a second sperm has entered and is dividing in the upper part of the egg; two more sperms which have almost entered the egg are seen at the top; ×42.

FIG. 12.—The fourth simultaneous free nuclear division; ×42.

FIG. 13.—At the bottom, 8 approximately equal nuclei are dividing; at the top, the division is also simultaneous, but the number of nuclei is large and they are irregular in size; there is a very marked polarity; ×42.

FIG. 14.—The nuclei of the lower half are large and are in the resting condition, while those of the upper half are smaller and are dividing simultaneously; the polarity is evident; ×42.

FIG. 15.—Evanescent segmentation: the nuclei of the lower part are larger and the cytoplasm is becoming vacuolated; ×42.

FIG. 16.—Many nuclei have migrated to the bottom and the cytoplasm of the entire proembryo is vacuolated; the ciliated band is still evident at the top; ×42.

FIG. 17.—Slightly later stage: many nuclei have amoeboid shapes; ×42.

FIG. 18.—Nuclei with dense cytoplasm at the base of the proembryo, just before the second free nuclear period; ×140.

FIG. 19.—Free nuclear division of the second free nuclear period; ×140.

FIG. 20.—Segmentation at the close of the second free nuclear period; ×140.

FIG. 21.—The cellular region is differentiated from the free nuclear region and there is some differentiation within the cellular region itself; ×100.

FIG. 22.—An outer layer of dermatogen-like cells has been differentiated; the dense cells just above are to produce the periblem, the plerome, and most of the suspensor; ×100.

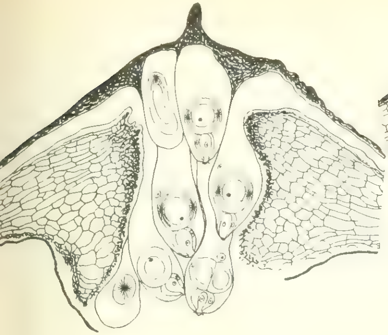
FIG. 23.—The upper cells of the cellular region are distinctly haustorial, and the slender tip, which has just broken through the egg membrane, is composed largely of haustorial cells; ×100.

FIG. 24. -Photomicrograph: upper part of fertilized egg, showing a part of the ciliated band and two sperms which have almost entered the egg; ×80.

FIG. 25. —Photomicrograph: first mitosis in fertilized egg, showing the mototic figure and the conspicuous kinoplasmic area about it; ×86.

FIG. 26.—Photomicrograph: one of the mototic figures of the fourth simultaneous free nuclear division; ×517.

FIG. 27.—Proembryo, showing the migration of nuclei toward the bottom; ×33.



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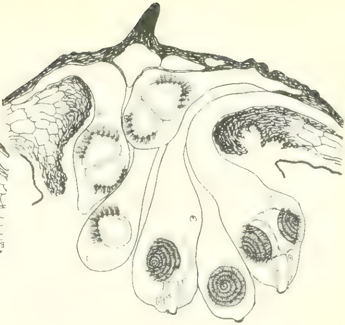

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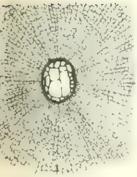

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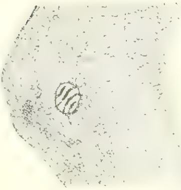

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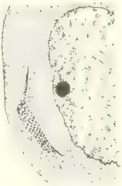

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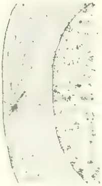

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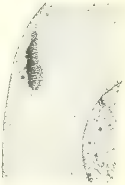

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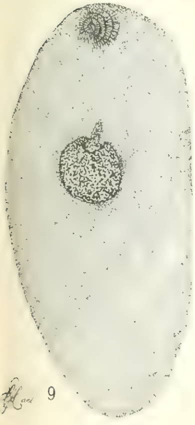

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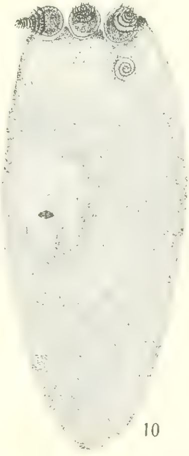

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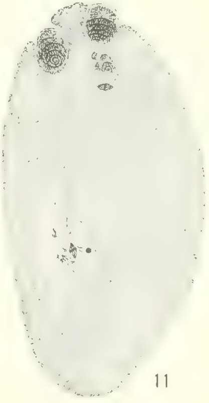

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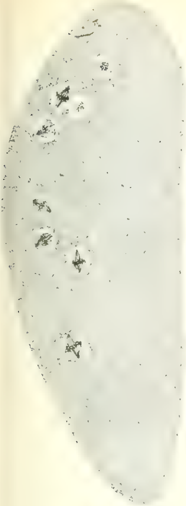
CHAMBERLAIN on STANGERIA



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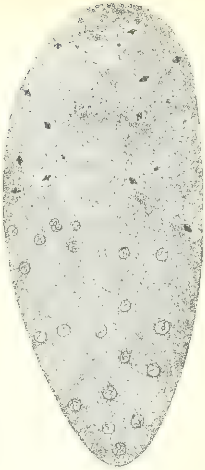

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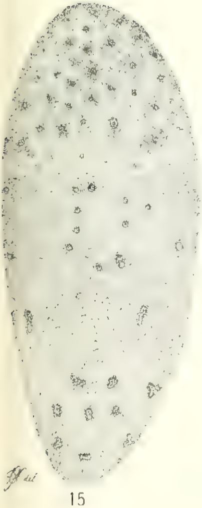

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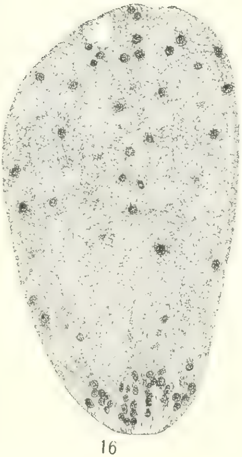

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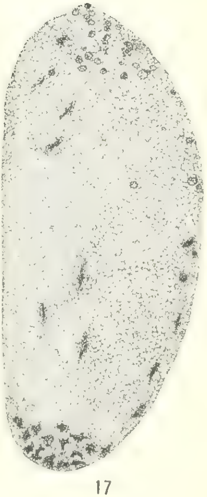

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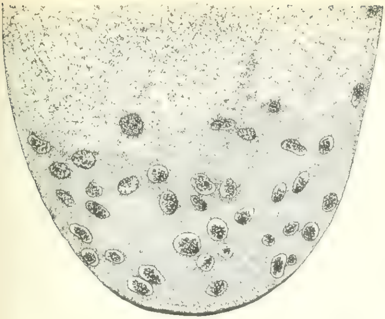

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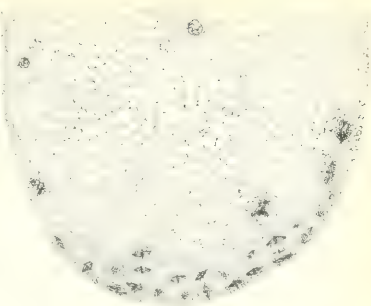

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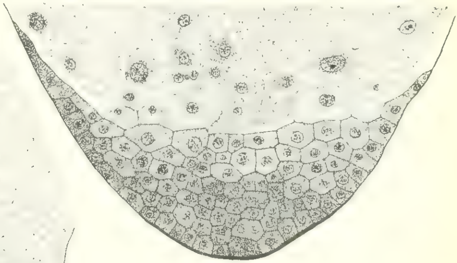

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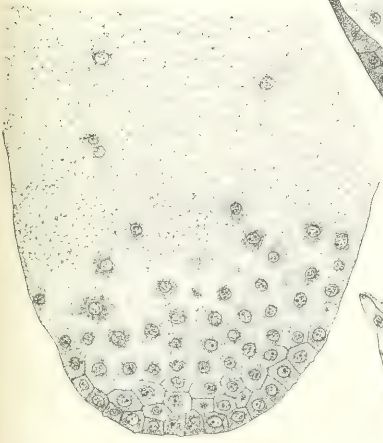

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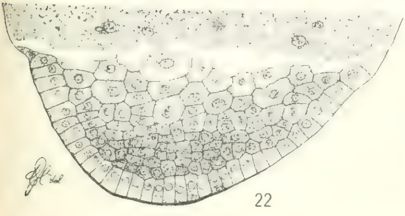

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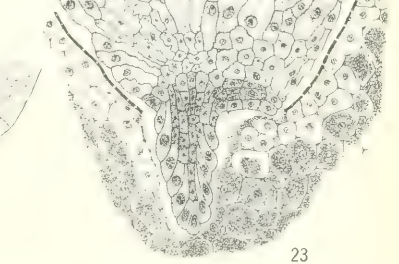

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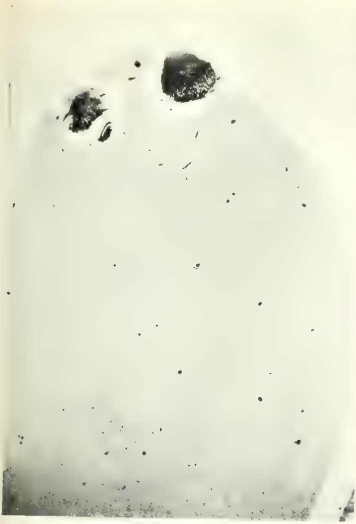

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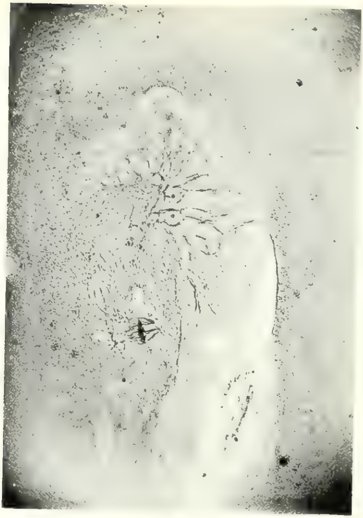

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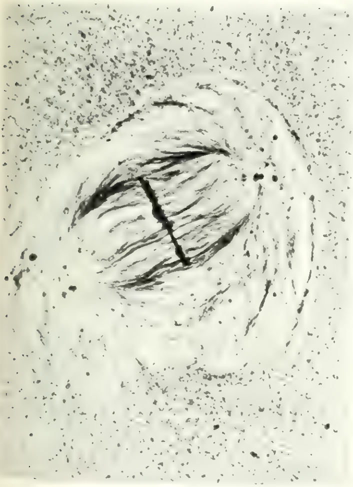

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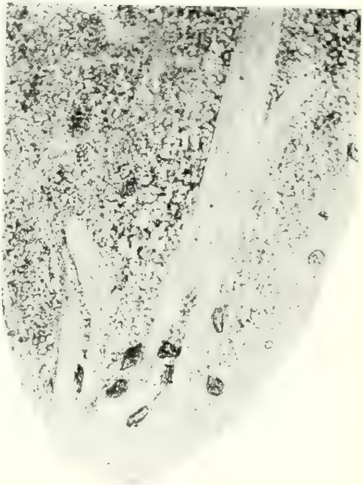

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CHAMBERLAIN on STANGERIA

REDUCTION DIVISIONS IN THE POLLEN MOTHER CELLS OF ALLIUM TRICOCCUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 215

WITH PLATES XXVIII-XXX AND ONE FIGURE

MILDRED NOTHNAGEL

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REDUCTION DIVISIONS IN THE POLLEN MOTHER CELLS OF ALLIUM TRICOCCUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 215

MILDRED NOTHNAGEL

(WITH PLATES XXVIII–XXX AND ONE FIGURE)

Introduction

In accordance with a suggestion of Dr. D. M. MOTTIER made while the author was attending Indiana University during the summer of 1914, material showing the reduction divisions of *Allium tricoccum* was examined with the idea of comparing it with the results of a previous investigation of *Allium cernuum* (**24**). In many stages the results were the same, but a further investigation of other phases of the spore mother cell development has forced the author to change some views previously held.

The nuclei are large and the haploid number of chromosomes is only 8; consequently, individual members or portions of the spirem can be traced through various stages with less difficulty than is usually encountered.

Literature shows a great diversity of opinion as to whether or not the chromosomes visibly retain their individuality throughout the resting period; also concerning the structure of the resting nucleus; the state of the chromatin as it enters the synaptic ball; the actual time and method of reduction; and the origin of the spindle fibers. These questions the author has attempted to answer.

JUN 3 1921

Material and methods

The material was collected along the steep, damp, shady banks of Clear Creek, 6 miles south of Bloomington, Indiana. Collections were made 4 days and 5 days apart, from June 30 to July 27, and while some of the anthers at the earlier date failed to show differentiation of sporogenous tissue, many from the later collections were in the shedding conditions. Often all stages from resting to tetrad would be found in a single umbel.

Strong chromo-acetic acid was used for killing and fixing, and allowed to act 36 hours, the fluid being changed once or twice during that period, after which the material was washed 18–24 hours by means of repeated changes of water, slowly dehydrated, cleared in chloroform, and then imbedded in 52° paraffin.

Sections were cut 5–12 μ thick, varying with the stages sought. For the spindle and in some instances for the spirem, Flemming's modified triple stain was found to be preferable, although for all other phases, especially the early prophases, Haidenhain's iron-alum-haematoxylin gave the best results, lichtgrün in clove oil occasionally being used for a counter-stain.

Description

PRESYNAPTIC AND SYNAPTIC STAGES

An attempt to determine the structure of the resting nucleus and the origin of the various stages that follow by first studying that resting condition would be difficult and indefinite. The investigation must begin with a stage concerning which there is little dispute, and from this may be traced the subsequent steps. Such a condition is to be found at late telophase of the last division in the sporogenous tissue, even though the chromosomes are more or less united by anastomoses, caused by considerable enlargement of the nucleus which followed the close association at early telophase (fig. 1). While still retaining this distinct individuality, although united with one another by anastomoses and at the same time slowly approximating end to end, a series of vacuoles appear along the median longitudinal portion of each chromosome (figs. 1–4), this being first made apparent by chromatin staining fainter along that portion (figs. 2, 3). In the beginning the vacuoles are short, narrow slits (figs. 2, 3), but later, owing to their enlargement,

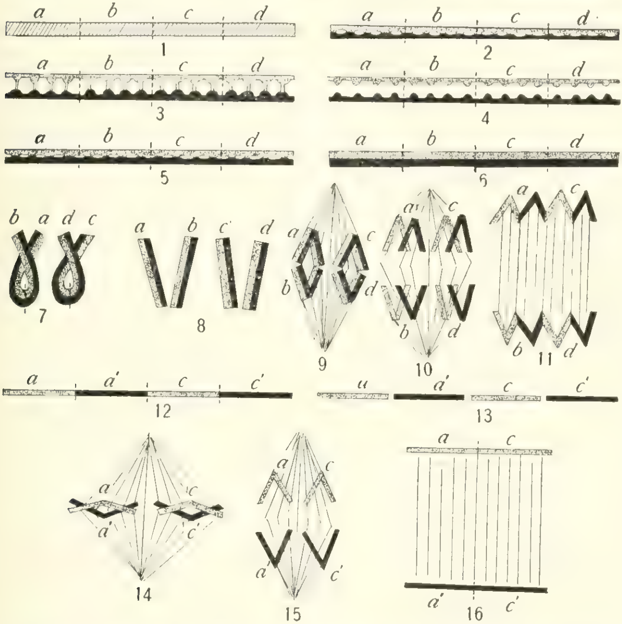

FIG. 1.—Diagram to illustrate reduction divisions as found in *Allium tricoccum*, 4 chromosomes only being shown: (1) telophase of last division of sporogenous tissue; (2) late telophase of same; (3) resting nucleus of pollen mother cell; (4) late resting condition; (5) going into synapsis; (6) condition of thread during synapsis and spirem; (7) segmented; (8) condition at time of third contraction and multipolar spindle; (9) metaphase; (10) anaphase; (11) telophase; (12) spirem of daughter nucleus; (13) segmented spirem of daughter nucleus; (14) metaphase; (15) anaphase; (16) tetrad or granddaughter nucleus.

the chromatin bordering the two sides of the vacuoles becomes more widely separated, although still joined together by portions or strands of chromatin that separate the vacuoles, resulting in each chromosome having a ladder-like appearance (figs. 2-8). The rate of vacuolization is by no means uniform, as will be seen from an examination of figs. 2-5, and the distribution of chromatin over the ladder thread is very uneven, being heavier at the junction of the sides and the connecting strands. During this process several nucleoli appear in the comparatively large open spaces of the nucleus, each large nucleolus containing a relatively large oblong vacuole (figs. 5, 8).

It must be kept in mind that the ladder structures with their two sides and connecting strands have arisen from single somatic chromosomes, and are the result of a series of vacuoles along their median longitudinal axis. The so-called typical resting stage consists entirely of these structures, therefore, still joined more or less with one another by the anastomoses that arose at the last telophase (figs. 5-8). In the very early prophase the sides of the ladder are very fine (figs. 3, 4), but as the chromatin material increases, the threads become heavier and more uniform (figs. 5-8), while at the same time the anastomoses break down, and the connecting strands of chromatin between the two parallel sides grow finer and finer (figs. 7, 8) until they entirely disappear, leaving as a result two parallel threads (fig. 8), which are daughter halves of single somatic chromosomes.

As the parallel spirems, now entering synapsis, contract and condense, they gradually approach each other and approximate, at times twisting about one another (fig. 9) and resulting in a single thick spirem (figs. 10-13) which is the condition of complete synapsis, the heavy thread appearing as a homogeneous structure twisted and coiled about itself (figs. 11, 12), causing the mass to have a lumpy, granular appearance (fig. 12). At no time during this period, when sectioning and staining have been the best, is the thread lost to view (figs. 9-14); for even at the time of the greatest contraction, portions of the thread may be traced across the mass (fig. 11). This step in development is not necessarily uniform, for portions of the spirem in synapsis have been observed showing the double nature distinctly (fig. 10).

While these activities are going on, the nuclear cavity as well as the entire cell enlarges; although most of the growth is after the chromatin material has contracted considerably. As the result of measuring many nuclei, only those being measured in which the entire nucleus was contained in the one section, the following statement may be made safely. Since the diameter of the contracted mass is considerably less than that of the nucleus just previous to contraction, or of the resting nucleus (figs. 7–12), synapsis is a true contraction. The synaptic mass lies almost or entirely against one side of the nuclear cavity, with the nucleolus just outside or partially held by a few strands of the spirem.

FORMATION OF BIVALENTS

As the synaptic mass loosens up, a comparatively thick, smooth thread may be seen twisting and winding about; and gradually loops free themselves, extending for various distances into the cavity (figs. 13, 14). It was stated that "a comparatively thick, smooth thread" was freed from the mass; while in fact the thread is irregular in outline and stains irregularly, the darker places being those denser chromatin aggregations seen in the ladder-like structure of the resting nucleus (figs. 3–9).

The spirem, although double in nature, is composed of single somatic chromosomes placed end to end, the double nature having arisen by the vacuolization or splitting of single chromosomes during late telophase of the last division of the sporogenous tissue.

When the spirem is entirely disentangled, loops of varying lengths are at first irregularly distributed within the nucleus (fig. 15); later, when they are peripherally arranged, the loops are longer and may be traced even entirely around the cavity, forming what is known as the hollow spirem (fig. 16). The double nature is rarely discernible during this latter period, since the thread has condensed and grown until it appears as a homogeneous structure staining uniformly throughout. Occasionally the halves of the thread separate (fig. 16) and may be seen even to twist about each other, the origin being in early synapsis (fig. 9, left side).

At the close of the hollow spirem stage the thread thickens and becomes entangled in the center (fig. 17), continuing until the typical second contraction results; which phase consists of the well known radiating loops that usually extend to the periphery (fig. 18). The loops represent somatic chromosomes end to end which separate at the outer bend; that is, at the curve away from the entangled mass (figs. 19, 20, 21) rather than at the lower bend. Each radiating loop, therefore, does not represent the bivalents as seen in figs. 21*x*, *y*, 22. Instead, one arm of a loop pairs with and twists about an arm of a neighboring loop, this being plainly evident in the material. Fig. 19 illustrates such a condition, for here a and a' were formerly a continuous loop, likewise b and b'; but, following separation, a and b twist about one another, then opposite ends in all probability form a loop, resulting in $a+b$ forming a bivalent, as seen in figs. 21*x*, *y*, 22, 23.

The bivalents of the aggregation continue to crowd together until only paired free ends extending from a dense mass can be recognized (fig. 20). When these segments loosen up, the chromosomes are found to be growing shorter and thicker. In case a misunderstanding should arise concerning the transition from fig. 21 to fig. 22, separate chromosomes have been drawn (figs. 21*x*, 21*y*). After the bivalents become dissociated and well distributed, eight are readily counted, each one representing 2 somatic chromosomes that formerly were end to end, although now they are twisted about each other. At times the 2 members of the bivalent are still continuous, that is, forming a loop; while in other cases they are not, causing the bivalent to be open at both ends. In either case it is not due to the splitting or to a separation of the halves of the spirem thread, since that spirem thread is composed of 16 somatic chromosomes with an end to end arrangement.

FROM THIRD CONTRACTION TO DAUGHTER NUCLEUS

About the time the bivalents are more or less distributed within the nuclear cavity, the fibers, which stain blue with Flemming's triple stain, appear outside the nuclear membrane (figs. 22, 23), running parallel to it, the ends extending out into the cytoplasm into which they merge (figs. 23, 24). While these are increasing in number and the nuclear membrane is disappearing, a peculiar behavior of the chromosomes has been noticed which seems to have a definite relationship to phases in the development of the spindle. The nuclear membrane slowly and unevenly disappears (fig. 23), this being accompanied by further growth of fibers. These fibers gradually appear within the nuclear space even before the membrane is entirely gone (fig. 24). When this activity first begins, the chromosomes move slowly toward the center, so that by the time of the stage shown in fig. 25, where the membrane has entirely disappeared, the bivalents are tightly massed in the center, forming a *third contraction*. Step by step the kinoplasmic fibers encroach upon the chromosomes (figs. 25, 26), until they come in contact with them (fig. 27), this being immediately followed by the loosening up of the aggregation (figs. 28, 29). By this time there is a strong multipolar complex, which is apparent as early as the disappearance of the membrane. The number of poles may be many and irregularly arranged (figs. 28–31), and as the fibers are rearranged into sharper points, the chromosomes become more and more dissociated (figs. 28–31) and scattered upon the fibers. Those bivalents which formerly consisted of the two end to end somatic chromosomes and formed a loop (fig. 22) have separated transversely, making 16 chromosomes or 8 pairs (figs. 29, 30, 30*x*). Fastened to each member of a pair, approximately at the middle, is a group of kinoplasmic fibers (fig. 30*x*) extending to one of the several poles. As the multipolar spindle changes to the bipolar shape, the chromosomes that formerly were scattered irregularly upon the fibers (figs. 29–32) gradually arrange themselves upon the spindle's equator (figs. 32–35). During this latter period the chromosomes shorten considerably, having reached their largest size during multipolar phase, as well as slowly untwisting.

The forms that the pairs may assume now are various; some remaining slightly twisted, others form a U, while still others may become linked with one another, forming an X, or the ends of the pair may remain in contact, forming a ring. No form was found to be conspicuously dominant (fig. 31). Polar views of the spindle first show the chromosomes not to be peripherally placed (fig. 32), although before metaphase is completed they take that position (fig. 33). At this time the chromosome count again can be taken without difficulty.

The individual fibers that are attached to each chromosome may become so closely associated at the point of union with them that the collection at first sight appears as a homogeneous mass and a part of the chromosome (fig. 34). These wefts retain their individuality for their full length and many times terminate in a very blunt pole (fig. 34).

During metakinesis, that is, just at the time when the paired segments separate, the dissociation of the two approximated halves, which originated in the resting stage (figs. 1–11), is finally completed (fig. 35), showing that the splitting or vacuolization of the somatic chromosomes in early prophase was in preparation for the homotypic division. There are now 16 chromosomes, for each half becomes entirely separated from its partner and does not again approximate (figs. 36–39). As the 16 members approach the poles, they shorten considerably, and as they crowd together an end to end formation results, thus forming an irregular spirem 16 chromosomes in length (figs. 37, 38). The crowding together continues until the entire chromatin mass is so closely associated that it is very difficult to distinguish the individual members; although, after the new nuclear membrane is formed, the looping spirem is again easily recognizable (fig. 39).

HOMOTYPIC MITOSIS

After the new membrane has been formed and the chromatin mass loosened up (fig. 39), anastomoses between portions of the looping spirem are everywhere evident. A coarse reticulum is formed as a result of the continual growth of the nucleus and the chromatin (fig. 40); but a resting stage is never reached. The spirem, which consists of 16 segments arranged end to end, will come directly from this reticulum (figs. 41, 42). This continuous spirem is quite regular in outline at first (fig. 41), but as the time for segmentation and formation of the multipolar complex approaches, the thread is more irregular and the outlines of the segments evident (figs. 42, 43); although it is not until the bipolar spindle is visible that the spirem separates into the 16 chromosomes (fig. 44). Simultaneous with this separation there is a pairing; so that 8 pairs of chromosomes come to lie upon the spindle fibers (figs. 44, 45). The paired chromosomes do not take the characteristic forms of the heterotypic mitosis, for at this time, although there is a pairing, there is no twisting about of the members; but, instead, the tendency is to assume more elongated forms, such as hooks, rods, or wide U's. One member of each pair passes to a pole, where again there is an end to end approximation, as in heterotypic mitosis, of the 8 chromosomes or the haploid number (fig. 49). The tetrad, or 4 granddaughter nuclei, each containing 8 chromosomes, may all lie in one plane, although at times they occupy two planes.

Discussion

PRESYNAPTIC AND SYNAPTIC STAGES

Since the investigation began with the examination of the late telophase of the last division of the sporogenous tissue while the individual chromosomes were still plainly evident, although joined together by anastomoses, it is clear to the author that there is not a pairing of somatic chromosomes in fig. 1, or that a previous approximation has taken place. If such were the case, 8 chromatin groups only would be visible. Even at this early phase the vacuoles are making their appearance along the median longitudinal line. The chromatin bordering these vacuoles could not be called linin, although the later enlargement of these forms the fine chromatin structure (figs. 2–8) that ALLEN (**1**), MOTTIER (**21, 22, 23**), STRASBURGER (**30, 31**), DIGBY (**7**), and others term linin. Furthermore, the heavier masses of chromatin granules are due to the nature of vacuole formation; for, as they enlarge, a greater amount of chromatin will be left at the angles between the vacuoles, thus giving rise to the so-called "chromomeres" strung along at irregular intervals on a linin thread.

BEER (**2**), MOTTIER (**22, 23**), and MOTTIER and NOTHNAGEL (**24**) find a single spirem formed from the network, in which condition it enters synapsis; while ALLEN (**1**), GRÉGOIRE (**16**), GRÉGOIRE and WYGAERT (**17**), BERGH (**3, 4**), YAMANOUCHI (**32, 33**), ROSENBERG (**27**), and OVERTON (**25, 26**) see a pairing of spirems either previous to or during synapsis, this act involving the pairing of somatic chromosomes, presumably maternal and paternal, after which they approximate and at metaphase of heterotypic mitosis separate. Nothing more than an inference or a suggestion has been found in the literature showing that the double nature of the spirem of the heterotypic mitosis is due to the splitting or vacuolization of somatic chromosomes in the early prophase. In a paper on *Galtonia* by Miss DIGBY (**7**), the split for the division at metaphase of the somatic mitosis is shown to arise by the vacuolization in early prophase, thereby forming the split or the double thread. A similar condition is found in *Vicia Faba* by SHARP (**28**), and by FRASER and SNELL (**12**). As the result of the conditions found, Miss DIGBY says: "By taking a broad and comparative view of this heterotypic prophase in relation to the somatic prophase, one is forced to admit that the parallelism of the one is homologous with that of the other"; although in her conclusion she states that "the parallel portions in both represent longitudinal halves of somatic chromosomes, and are probably sister halves of the same chromosome, which are now severally coming together and condensing to form the somatic or univalent chromosome." The series of drawings are incomplete at this critical period, and any conclusion would have to be based upon her drawings 39*a* and *b*, and 40, none of which is later than the writer's figs. 3 and 5. Furthermore, steps illustrating the origin of these figures from figs. 30 and 31 have not been shown, and the gradual transition from the "beaded" resting nucleus (DIGBY **7**, figs. 33, 36, 37) to the double condition of the spirem after synapsis appears to be more of a theory or an inference than a statement of observed facts.

In the early prophases of the heterotypic mitosis of *Vicia Faba*, Miss FRASER (**11**) finds conditions corresponding to those observed in *Allium tricoccum*. In this paper she describes diamond-shaped meshes that are due to the splitting of the somatic chromosomes in the early prophases; then later, that is, in synapsis, the cross-connections between the meshes breaking down, thus forming the spirem; and finally, in early anaphase, each chromosome splitting preparatory to homotypic mitosis, the origin of the split having been seen in the diamond meshes. The idea is very similar to that described by the author, but as in the paper by Miss DIGBY (**7**), Miss FRASER fails to have a series sufficiently close to demonstrate the origin of the split and the formation of the spirem from these meshes. While the resting nucleus is said to consist of diamond-shaped meshes, it is in all probability the same as the ladder-like formation of *Allium tricoccum.*

As has been previously stated, the number of chromosomes in *Allium tricoccum* is small and the size large, so that the difficulty so often encountered in following the development is considerably lessened. Throughout these critical phases every precaution has been taken to prevent overlooking important stages. Since the pollen mother cells at the upper end of a loculus are a little earlier than those at the lower end in development, at least two consecutive stages could be found in a single section. In every case the later of the two stages observed has been found in the upper end of the loculus, where the third has been found and drawn, decreasing to a great extent the possibility of omitting critical stages or placing the wrong interpretation upon the origin of the double character found in the resting nucleus. Had figs. 5, 7, or 8 been the first nuclei observed after the telophase of the previous division, the conclusion could readily be drawn that the double thread arose by the pairing of somatic chromosomes; but, after seeing the beginning of vacuolization (figs. 1, 2) and following its development step by step (figs. 2 9), no other conclusion is possible than that the paired threads going into synapsis are the two halves of single somatic chromosomes, and not paired somatic chromosomes, as held by GRÉGOIRE (**16**) and YAMANOUCHI (**32, 33**).

Fig. 8 illustrates the breaking down of the connecting strands between the two sides of the ladder-like structures, and on the right side of this figure the act has been completed, leaving the halves completely separated except for the portion connecting them at the end. MOTTIER (**22**) states that "the delicate threads joining the chromatic masses may be found lying close to each other and parallel, but this does not signify that a double spirem is in process of formation"; although in his figures of this stage (MOTTIER **22**, figs. 1, 17, 34) he shows nuclei very similar to that in fig. 5 of *Allium tricoccum;* and by tracing this further it is very probable that the origin of the double nature of the hollow spirem, as described by MOTTIER (**21, 22, 23**), might be found.

No suggestion of the spiral arrangement with the strands radiating from a "Chromatinknoten" as described by BONNEVIE (5) has been observed.

That the chromosomes retain their individuality and do not break up into a network is claimed by GRÉGOIRE (15), YAMANOUCHI (32, 33), SHARP (28), OVERTON (25, 26), STOUT (29), BONNEVIE (5), and LAWSON (19); this is also evident in *Allium tricoccum*. Although the chromosomes become considerably vacuolate and thus cause the net appearance, the individual members never lose their entire individuality, as can be seen from the description and drawings, and later, owing to a larger amount of chromatin material and a more even distribution of it, the chromosomes form a more or less continuous thread, the spirem.

Little growth occurs during the later stages of the resting nucleus, but when the chromatin mass starts to contract, it increases slightly in size, but not to the extent claimed by LAWSON (19); that is, that the appearance of the contracted mass is due to the growing away of the nuclear membrane. Comparison of figs. 8, 9, 10, and 11[1] will make this clear, as fig. 11 is a drawing of an entire nucleus, the dimensions of the chromatin mass being considerably less than that of figs. 7 or 8. It is not until the mass has contracted extensively that the large increase in size of the nuclear cavity occurs (figs. 10, 11). Were it but an apparent contraction, as LAWSON (18) states, due to the inflow of karyolymph into the nuclear cavity, the osmotic pressure would be decreased, not increased, and the increase in size would be the result of the larger amount of fluid that it must hold.

As synapsis approaches, the two halves of the spirem gradually approximate (figs. 9, 10, 11), the final step usually being accomplished during synapsis (fig. 11); although even as late as the stage shown in fig. 10 the act might not have been completed along the entire length. This process corresponds to that described by GRÉGOIRE (14, 15), YAMANOUCHI (32, 33), OVERTON (25, 26), and ALLEN (1), although these investigators claim it to be the approximation of whole somatic chromosomes, or pseudo-reduction.

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[1] Figs. 1–12 were made with a magnification of 3500, while fig. 12 has a magnification of 2050, so that comparison of the latter with the former is not to be made where questions of size are concerned.

FORMATION OF BIVALENTS

With close observation during early spirem, the double nature is still discernible, although it is rare that the two halves separate as found in *Allium cernuum* (Mottier and Nothnagel **24**), and as commonly found in *Lilium*. The lumpy condition of the thread at this time is due to the larger chromatin collections of the early prophase (figs. 5-9), this appearance being the basis of the statements of Farmer and Moore (**9**), Mottier (**22**), and others who interpret such as dividing chromomeres, thereby initiating the longitudinal split. This dual nature has been seen in most cases, although it has been attributed to two sources. Those believing in pseudo-reduction in early prophase assert that it is the two spirems that have paired, while Mottier (**21, 22, 23**), Strasburger (**30, 31**), Beer (**2**), Gates (**13**), Farmer and Moore (**9**), and Farmer and Shove (**10**) say that the spirem has split in preparation for the homotypic division. In this latter case, however, the split was not traced to its origin. The two halves soon approximate so closely that the spirem appears as a homogeneous structure (figs. 15, 16). Comparatively few ends are seen when sections are cut 12 μ thick, and from all of those observed it appeared to be due to cutting. So far as the stages to follow are concerned, it would make little or no difference whether the spirem be continuous or non-continuous.

At first, as formerly stated, the spirem is irregularly placed within the nuclear cavity (figs. 14, 15), this being followed by a peripheral arrangement, thereby forming the typical hollow spirem as described by Mottier (**21, 22**), Mottier and Nothnagel (**24**), and Beer (**2**); at which time the thread may be followed for a considerable distance. As it thickens and shortens, the second contraction period is entered upon, although Overton (**26**) fails to find such a stage in *Thalictrum purpurascens*, *Calycanthus floridus*, and *Richardia africana;* also Grégoire (**16**) fails to observe it at times in *Lilium speciosum;* both regarding such an act as of little significance in the reduction division. The author believes this step to be of considerable importance.

Up to and including the second contraction, a nucleolus is usually present (figs. 17, 18), although some of the drawings fail to show such, as it was either not included in that section, or it was purposely left out, owing to obscuring too great a portion of the other chromatin material.

During second contraction (fig. 8), the characteristic radiating loops extend from the tangled mass, the first sign of segmentation being seen at the peripheral end of the loop, resulting in the free ends being next to the nuclear membrane (figs. 19, 20). ALLEN (**1**) has reported a similar observation in *Lilium canadense*, but this is contrary to the reports of MOTTIER (**21, 22, 23**) and BEER (**2**), these investigators describing the radiating loop as forming the bivalent. With the loop segmenting at its outer bend, it necessarily follows that either the bivalent is continuous at the lower end, as stated in the description of this stage, or that segmentation occurs at both ends, followed by a pairing of single somatic chromosomes. During the period of segmentation and formation of bivalents, the chromatin thread contracts, although not suddenly (cf. figs. 18–24), resulting in 8 thick bivalents being fairly evenly distributed within the nuclear cavity.

All cytologists agree upon the point that in heterotypic mitosis a bivalent consists of 2 somatic chromosomes, but concerning the mode of formation there is a great difference of opinion. ALLEN (**1**), BERGH (**3, 4**), GRÉGOIRE (**16**), GRÉGOIRE and WYGAERT (**17**), OVERTON (**25, 26**), ROSENBERG (**27**), and YAMANOUCHI (**32, 33**), who claim there is a pairing of somatic chromosomes or spirems in early prophase, state that the bivalent is composed of 2 segments that in the spirem were side by side, reduction therefore occurring by the pairing of 2 spirems; while BEER (**2**), FARMER and MOORE (**9**), FARMER and SHOVE (**10**), and MOTTIER (**21, 22, 23**) demonstrate that it is formed by the twisting about of 2 somatic chromosomes that previously were end to end in the spirem, thus causing a transverse segmentation to be responsible for the reduction. *Allium tricoccum* confirms this latter view, and if the series here given be followed, it will be seen that the arms *b* and *a* of fig. 19 have not arisen from the separation of 2 approximated spirems of a previous stage (fig. 18), yet doubtless these 2 will by a gradual thickening (figs. 20, 21, 21*x*, *y*, 22) form a bivalent (fig. 22).

Each bivalent, therefore, is composed of 2 somatic chromosomes, presumably maternal and paternal, that previously had an end to end arrangement in the spirem, and which may be open either at both ends or at one end. In the latter case a later segmentation will separate the two. So far as any result that is to follow is concerned, the author conceives it to be of little importance whether it be the one condition or the other. Some claim that the bivalents are always open at both ends, which would necessarily be the case were they derived from a paired spirem; but definite cases have been found where the bivalents were continuous at one end (figs. 22, 23), and in fig. 22, lying under another bivalent, will be seen one in which the two arms have not twisted about each other as yet, but are lying more or less stretched out in the cavity. Under such circumstances the bivalent could not have been formed as GRÉGOIRE (**16**), ALLEN (**1**), and YAMANOUCHI (**32, 33**) have claimed.

To repeat once more, each arm of a bivalent is necessarily of a double nature, owing to the approximation of the two halves of single somatic chromosomes in synapsis, so that in cross-section a bivalent has a tetrad arrangement.

FORMATION OF SPINDLE AND DAUGHTER NUCLEI

Nothing has been found in the literature describing the third contraction or its relationship to spindle formation.

LAWSON (**18, 20**) finds the weft of fibers, these being transformed cytoplasm, outside the nuclear membrane as early as the spirem stage, although in *Allium tricoccum* they are not visible until segmentation.

After carefully studying the paper entitled "Nuclear osmosis as a factor in mitosis" by LAWSON (**20**), and then comparing the same with results found in *Allium tricoccum*, many points of disagreement were encountered. As has been pointed out by FARMER (**8**), LAWSON has used the term "permeable membrane" in describing the nuclear membrane, after which he continues to speak of osmotic systems and exosmosis. In this discussion, when speaking of the membrane in this connection, the term "semipermeable membrane" will be used. Undoubtedly the nuclear membrane, which is the inner limiting layer of the cytoplasm formed there by the contact of cytoplasm and karyolymph, is a semipermeable membrane, and also, at the time of spindle formation, exosmosis is taking place, since the nuclear cavity is gradually decreasing in size (figs. 22–27) from the time of development shown in figs. 22 and 23, when the fibers are first evident outside the membrane. Since the membrane results from the contact of cytoplasm and karyolymph, the same chemical reaction would occur when the nuclear sap gradually diffuses into the cytoplasm. Such is the author's interpretation of the weft of fibers outside the nucleus. The diffusion is gradual, and the precipitation would then be slight, resulting in the fine fibers. The cytoplasm is not forced to occupy more cubical space, as LAWSON claims, for karyolymph is steadily, though slowly, passing through this semipermeable membrane, and owing to the precipitation the fibers occupy the space left by the receding nucleus.

It is while the foregoing is occurring that the third contraction or massing of the chromosomes in the center of the cavity becomes so conspicuous (figs. 23–27), although as yet the fibers are not in contact with them; and furthermore, the membrane disappears previous to the filling up of the cavity by the fibers (figs. 24 26). If the plasma membrane completely invested the bivalents, as claimed by LAWSON, a cavity would be left between it and the fibers in the case of *Allium tricoccum* (figs. 25, 26), under which circumstances the fibers could not be moored to the membrane (LAWSON **20**).

It was owing to the contact of the two chemically different substances that the heavy plasma membrane was precipitated, but with the kinoplasmic fibers now being formed between the membrane and the reticulate cytoplasm, this chemical antithesis is decreased, resulting in a decrease in the amount of precipitation. In other words, the membrane becomes thinner and thinner until it either disappears entirely or becomes a part of the spindle fibers. When the nuclear cavity is completely filled with the kinoplasmic threads, the chromosomes loosen up, probably owing to the completion of exosmosis from the cavity, as well as partially on account of the osmotic systems within each chromosome.

The bivalents are now open at both ends and not quite as large as formerly, decreasing from now on (fig. 24). When they first loosen up, fibers are not fastened to each chromosome apparently; but as the multipolar complex forms, a weft becomes moored to each one, in many instances so conspicuous (figs. 29–35) that it appears to be homogeneous structure at the point of attachment, owing to the thick fibers and also to their close association.

Each chromosome, as stated by LAWSON (**20**), is saturated with karyolymph and is an osmotic system; although this does not necessarily mean that the old membrane must surround each member in order to accomplish this. If such were the case, the membrane would have to break up into the proper number of pieces, wrap about each chromosome, and then become sealed; a process which is far more complex than ever before attributed to a nucleus. Each chromosome will have the power to develop its own membrane, owing to diffusion of the sap from it, and since each then will have the same osmotic power as the nucleus did as a whole, the same process will continue as it did with the nucleus, resulting in the formation of a weft of fibers from each individual. The fact that these wefts are distinct from the other fibers points strongly toward this idea.

LAWSON'S theory that the shift from multipolar spindle to bipolar spindle is an expression of "lines of tensions" appears to the author to have little or no foundation, since the reticulate cytoplasm is not forced to occupy less cubical space than formerly.

The paired chromosomes, which formerly were tightly twisted about each other, gradually come to lie upon the equatorial plate; the fibers formed outside the nuclear membrane extending from pole to pole and the individual tufts of fibers attached to the chromosomes extending to but one pole. At metaphase the separation of whole somatic chromosomes, that previously were end to end in the spirem, is completed, this reduction being immediately followed by the longitudinal separation of the halves (fig. 35) of each of these, the origin of which was seen in the resting nucleus (figs. 2–10). Those believing in the pairing of somatic chromosomes or spirems in early prophase or pseudo-reduction have little to say concerning the origin of this split; while those claiming that the spirem is a single structure splitting during the spirem stage demonstrate that the separation during anaphase is the result of this former activity.

Contrary to most reports, the halves of the chromosomes in *Allium tricoccum* become entirely dissociated during anaphase, so that a polar view of such shows 16 individuals, or the $2x$ number. These 16 remain distinct from now on, and at late anaphase or early telophase they join up end to end (fig. 38), after which the looping spirem becomes very much crowded. As some karyolymph still remains, this diffuses out and the nuclear membrane is formed about the densely crowded chromatin mass, as described by LAWSON (**20**) and YAMANOUCHI (**32**).

HOMOTYPIC MITOSIS

A spirem consisting of 16 segments, that approximated end to end at late anaphase of the previous mitosis (fig. 38), forms early in the daughter nucleus (fig. 42), since a resting condition does not intervene. In all reports read concerning the homotypic divisions, the spirem is interpreted as being x chromosomes in length and double, owing to the longitudinal separation during the previous anaphase. As the multipolar spindle appears, the spirem forms 16 segments (fig. 44) which immediately pair (fig. 45), forming 8 pairs of half chromosomes. It is probably owing to the rapidity of the pairing that previous investigators have claimed the longitudinal split to be completed at this time instead of during the heterotypic mitosis. Unless a close series had been followed and the end to end approximation of the half chromosomes been observed, the author would probably have made a similar interpretation. If each chromosome is to maintain its individuality, no other results could be expected.

All this goes to show that at metaphase of heterotypic division there is a reduction or separation of characters, but it is not until homotypic division that the reduction in number is actually accomplished.

Conclusion

In comparing the nuclei during the reduction division, as seen in *Allium tricoccum*, with the nuclei during somatic division, as described by SHARP (**28**) for *Vicia Faba* root tips, it is seen that both have a similar structure during the early prophase. The first difference is evident when, apparently owing to some osmotic force, the thread contracts into the synaptic ball. During this contraction there is an increase in chromatin substance, preparatory to the two rapid divisions which follow. At the time of second contraction, this condition undoubtedly being due to osmotic activity again, bivalents are formed. Following the third contraction, where it has been pointed out plainly that exosmosis is the factor underlying spindle formation and massing of bivalents, the members of each pair separate for opposite poles. Seemingly this last contraction, or the 3 contractions taken together, hold in check the dissociation of the halves, which in somatic mitosis would have occurred at this time; for immediately following the separation of whole chromosomes, the halves move apart, join end to end, and, as soon as a new spindle is formed, go to opposite poles.

From this investigation it appears to the author that on account of these various contractions, a regular somatic mitosis, although started normally, is first varied and then checked for a time, resulting in the heterotypic division, and not until homotypic division is the typical mitosis completed.

Summary

1. During late telophase of the last division of the sporogeneous tissue a row of vacuoles appears along the median longitudinal axis of each chromosome, these enlarging until each member is a ladder-like structure. Accompanying this there is an end to end approximation. Such is the condition of the resting nucleus.

2. The paired threads entering synapsis and there approximating are the two sides of the ladder, the connecting strands having broken down. This process does not represent the pairing of two spirems. Throughout this period the chromosomes have retained their individuality.

3. The spirem, which consists of 16 end to end chromosomes, will take the form of radiating loops during second contraction, segmentation occurring at the outer bend. Each of the bivalents so formed consists of two somatic chromosomes that were end to end in the spirem.

4. Spindle fibers are the result of exosmosis of karyolymph into the cytoplasm, these being formed after the same fashion as the nuclear membrane. The membrane, if it persists, will be a part of the fibers.

5. The third contraction which accompanies fiber formation consists of a balling up of the chromosomes previous to the complete filling up of the cavity with fibers.

6. Each chromosome is an osmotic system in itself and capable of forming its own weft of fibers after the sap from the nuclear cavity has been exhausted.

7. The heterotypic division or the reduction of characters results from a transverse separation of whole chromosomes.

8. During early anaphase the halves of the chromosomes, which originated in presynapsis, separate longitudinally and at early telophase approximate end to end, forming the looping spirem of the daughter nucleus, $2x$ chromosomes in length. Division in homotypic mitosis, therefore, results originally from a longitudinal separation.

9. The transverse separation of the 16 segments during early metaphase of the homotypic mitosis is immediately followed by their pairing.

10. To all appearances a typical mitosis is begun, but is varied and delayed for a time by a heterotypic mitosis as the result of the various contractions, being finally completed in the homotypic division.

To Dr. J. M. COULTER and Dr. C. J. CHAMBERLAIN I wish to express my appreciation for their most helpful suggestions and criticisms during the progress of this work, and also to Dr. D. M. MOTTIER of Indiana University, under whom this work was commenced.

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EXPLANATION OF PLATES XXVIII–XXX

All figures were drawn with the aid of a Spencer camera lucida with Bausch and Lomb $\frac{1}{12}$ immersion and ocular 12, except figs. 1–11 inclusive, which were drawn with Bausch and Lomb $\frac{1}{16}$ immersion and ocular 12. Magnification of figs. 1–11 inclusive ×3500; all others ×2650. The plates are reduced to two-thirds the original size.

PLATE XXVIII

FIG. 1.—Late telophase of the last division of the sporogenous tissue, showing the anastomoses and the beginning of vacuolization along the median longitudinal axis.

FIG. 2.—A later stage, most of the chromosomes being vacuolate.

FIGS. 3, 4.—Early stages of the pollen mother cells, which consist of ladder like structures that arose by vacuolization of the chromosomes; some of the chromosomes still entire, owing to unevenness of vacuolization.

FIG. 5.—Typical resting nucleus of the pollen mother cell; chromatin aggregations comparatively large, and the connecting strands very fine.

FIGS. 6, 7.—Somewhat later stages in which the anastomoses are disappearing, connecting strands between the parallel sides becoming very fine in structure, and the sides of the ladder growing more uniform.

FIG. 8.—Late resting stage; the connecting strands disappearing, leaving two parallel daughter spirems.

FIG. 9.—Early synapsis; the two parallel daughter spirems approaching each other and at places approximating.

FIG. 10.—A later stage; the double nature still plainly discernible in many places.

FIG. 11.—Complete synapsis, showing the coiled spirem in which approximation has been completed.

FIG. 12.—Same as above, but magnification not so great.

FIG. 13.—Coming out of synapsis.

FIG. 14.—Late coming out of synapsis, the double row of granules still visible, these being the remains of the larger chromatin aggregations as seen on the ladder-like structures.

FIG. 15.—Spirem; traces of the granules of early prophase showing.

FIG. 16.—Hollow spirem; granules evident and occasionally a split where approximation was not complete.

PLATE XXIX

FIG. 17.—Beginning of second contraction; the spirem very heavy.

FIG. 18.—Second contraction consisting of radiating loops.

FIG. 19.—Segmentation beginning at the outer bend of the loop; *a* and *a'* formerly being continuous, likewise *b* and *b'*; although at this time *a* and *b* are twisting about each other to form the bivalent.

FIG. 20.—Further contraction of the chromatin mass in which the free ends of the bivalents are radiating out from the mass.

FIG. 21.—Loosening up of the bivalents.

FIG. 21*x* and *y*.—Showing the gradual thickening of the bivalents.

FIG. 22.—Eight bivalents evenly scattered within the nuclear cavity in which some are seen to be open at both ends, others closed at one end, while still another, lying under the others, is seen to be stretched out instead of twisted.

FIG. 23.—Beginning of the fiber formation outside the nuclear membrane which accompanies the third contraction; also the disappearance of the nuclear membrane.

FIG. 24.—A slightly later stage; nuclear membrane apparently gone on one side of the nucleus.

FIG. 25.—Later; the fibers in contact with part of the chromosomes; nuclear membrane entirely disappeared; the multipolar complex begun.

FIG. 26.—A later stage.

Fig. 27.—Third contraction complete; fibers have completely filled the nuclear cavity.

Fig. 28.—Multipolar spindle; chromosome aggregation loosening up.

Fig. 29.—Formation of the individual wefts of fibers for each chromosome; the scattering of the bivalents upon the fibers; and a step farther in the transition from multipolar to bipolar spindle.

Fig. 30.—A later stage.

PLATE XXX

Fig. 31.—Bipolar spindle; bivalents stretched out upon the fibers.

Fig. 32.—Polar view of early metaphase.

Fig. 33.—Polar view of metaphase with bivalents peripherally arranged.

Fig. 34.—Metaphase in which the individual wefts of fibers are extending from each chromosome to the pole; also the fibers' massive organization at the point of attachment; to the left is a weft that has been broken from its chromosome.

Fig. 35.—Early anaphase; the longitudinal halves of each somatic chromosome that arose by vacuolation in early anaphase are again becoming apparent.

Fig. 36.—Anaphase; halves of the somatic chromosomes entirely separated.

Fig. 37.—Polar view of the same.

Fig. 38.—Late anaphase; 16 half chromosomes approximating end to end to form a spirem $2x$ chromosomes in length.

Fig. 39.—Daughter nucleus after loosening up of chromosomes.

Fig. 40.—Daughter nucleus; irregular net appearance due to the anastomoses.

Fig. 41.—Daughter nucleus; spirem stage in which the fibers of previous mitosis have not disappeared.

Fig. 42.—Daughter nucleus; late spirem in which the outlines of the segments are discernible.

Fig. 43.—A later stage in which the multipolar complex has appeared.

Fig. 44.—Segmentation into 16 segments followed immediately by their pairing.

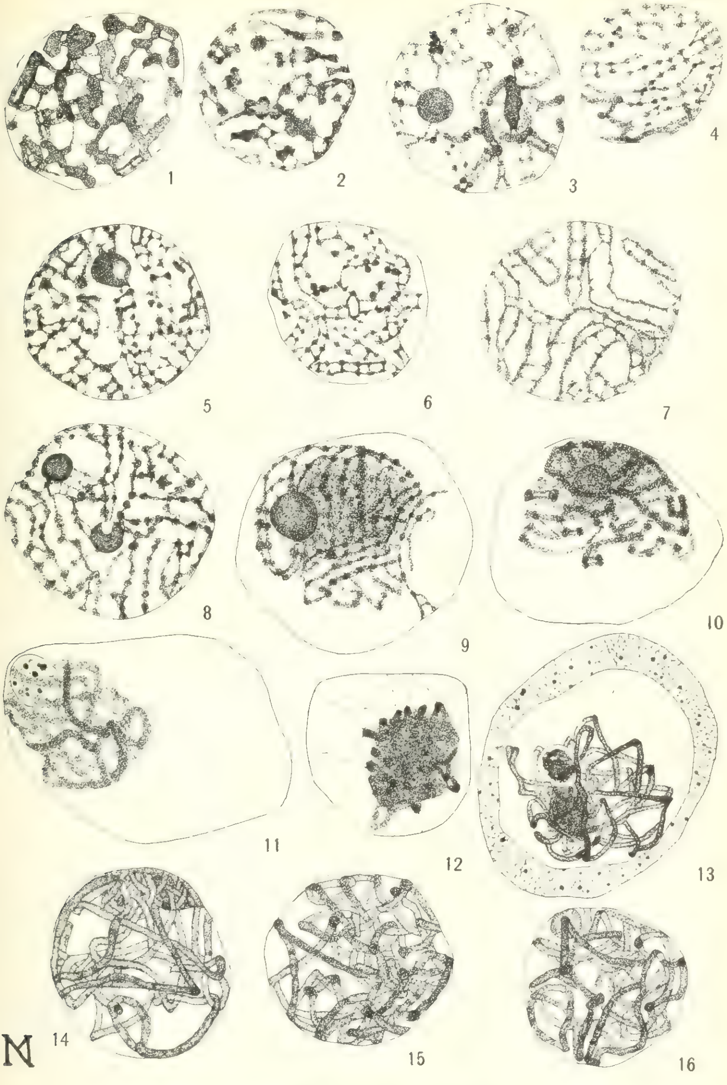
Fig. 45.—Polar view of metaphase of homotypic mitosis.

Fig. 46.—Late metaphase of the two daughter nuclei.

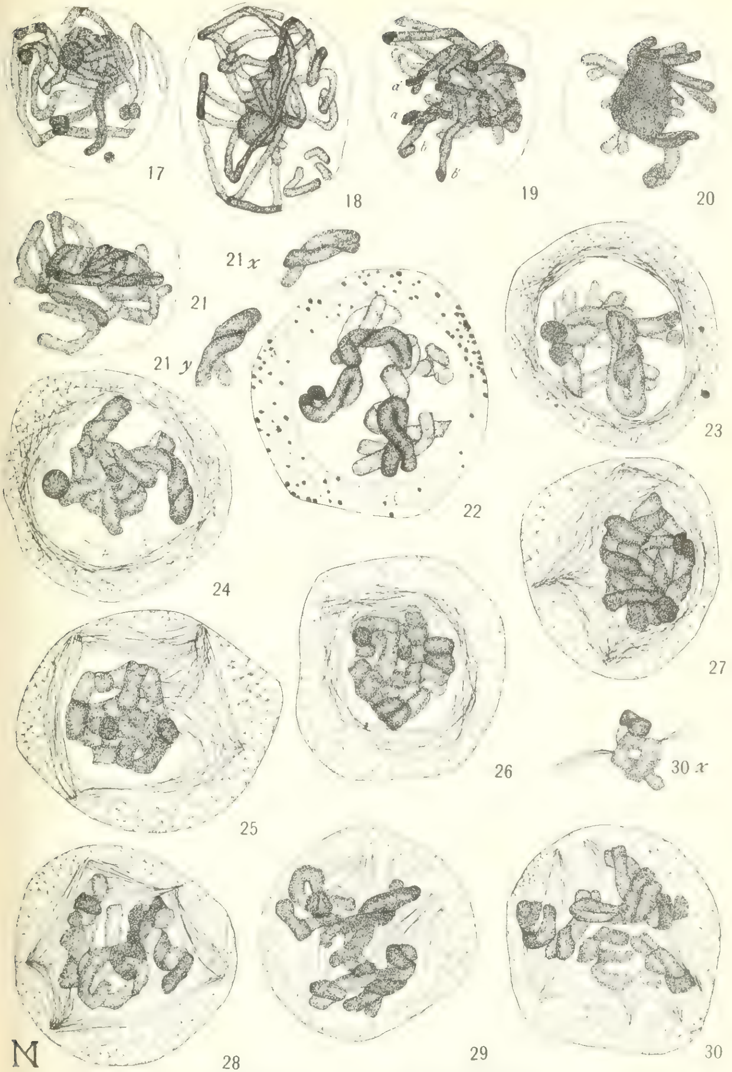
Fig. 47.—Same, but only one daughter nucleus in plane.

Fig. 48.—Anaphase of homotypic mitosis.

Fig. 49.—Telophase of homotypic mitosis; end to end approximation of 8 chromosomes just previous to organization of granddaughter nucleus.



NOTHNAGEL on ALLIUM TRICOCCUM



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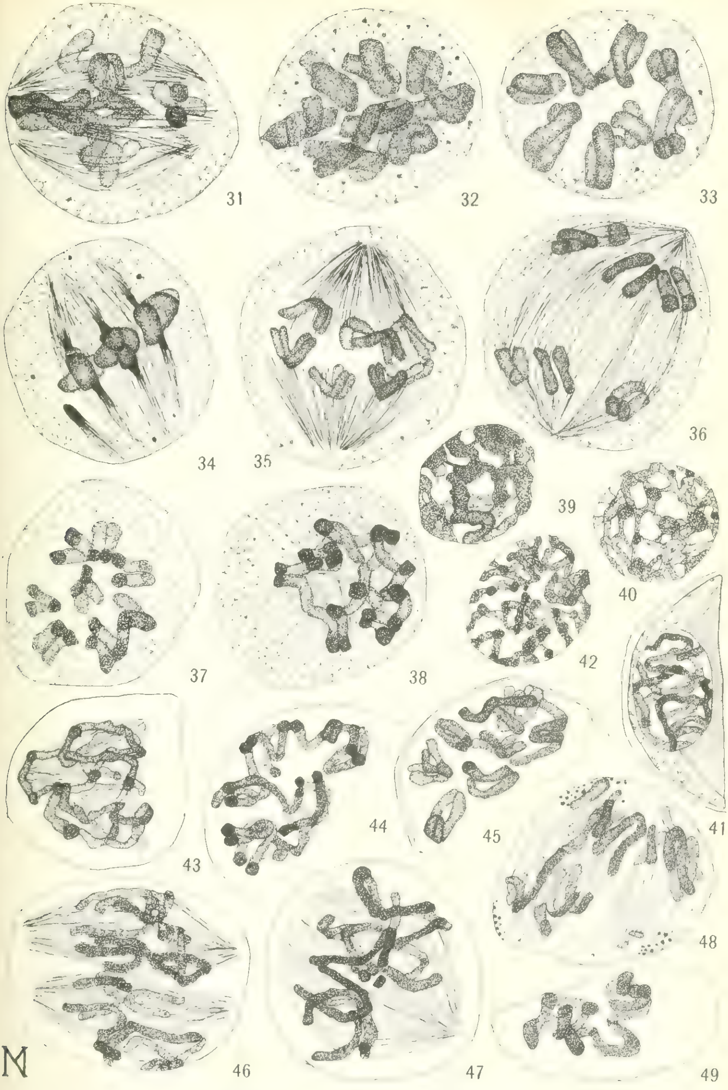

NOTHNAGEL on ALLIUM TRICOCCUM

With my sincere regard

Dr. Britton, — Earl E. Sh

STUDIES IN THE GENUS BIDENS. III

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 216

(WITH PLATE XXXI)

EARL E. SHERFF

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STUDIES IN THE GENUS BIDENS. III

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 216

EARL E. SHERFF

(WITH PLATE XXXI)

Bidens attenuata, sp. nov.—Herba (annua?), +4 dm. alta; caule et ramis hispido-tomentosis, subtenuibus, subperspicue striatis, teretis (nisi caule infra angulato). Folia opposita (aut superiora alternata), petiolata, petiolo adjecto 1.5–6 cm. longa, bi- aut tri- (aut etiam quadri-) pinnata, subsparsim adpresso-hispida, hispido-ciliata; ultimis lobulis integris, linearibus, indurato-apiculatis, saepe convolutis, 0.3–0.7 mm. latis. Petioli 3–11 mm. longi, hispidi, ad basim connati. Capitula terminalia, ligulata, longe et tenuiter pedunculata, pedunculis 5–15 cm. longis. Involucrum basi hispidum; squamis duplici serie dispositis; exterioribus (circ. 6) linearibus, glabratis aut sparsim hispidis, 3–4 mm. longis; interioribus dimidio longioribus, dense hispidis, margine diaphanis. Ligulae flavae, striatae, elliptico-ovatae, ad apicem subdentatae aut integrae, 1.8–2 cm. longa. Paleae lineares, margine diaphanis. Achaenia (submatura) linearia, glabra, costata, biaristata aristis retrorsum hamosis (3–6 glochidiis), 5 mm. longa.

Ghiesbrecht, Chiapas, Mexico (type in Herb. Gray).

ASA GRAY had labeled this plant in the herbarium "*B. ferulaefolia* var.," but the true *Bidens ferulaefolia* DC. (*Coreopsis ferulaefolia* Jacq., Hort. Schoenb. 3:65. *pl. 375.* 1798) is a very different plant.

Bidens Andrei, sp. nov.—Herba (ad basim suffrutescens?), 1–2 m. alta, glabrata, caule tetragono et striato; ramis tetragonis, striatis, (superioribus) irregulariter arcuatis et in nonnullos ramulos (aut pedunculos) divisis. Folia opposita, petiolata, petiolo adjecto 2.5–7 cm. longa, bi- aut tri- (aut quadri-) pinnata, supra minute et non dense adpresso-hispida; ultimis segmentis linearibus, integris aut lobulatis, indurato-apiculatis. Petioli 0.3–2 cm. longi, basi connati. Capitula multa, tenuiter pedunculata, pedunculis 4–12 cm. longis, 0.4 cm. (ad anthesin) –1 cm. (in fructu)

SEP 1 Given by N. L. BRITTON.

alta, ligulata. Involucrum basi plus minusve hispidum; squamis duplici serie dispositis et demum reflexis; exterioribus (circ. 6) linearibus, apiculatis, subsparsim pubescentibus, 1–2 mm. longis; interioribus lanceolatis, membranaceis, 2–3 mm. longis, margine diaphanis. Ligulae (6 aut pauciores) lanceolatae, 4–5 striatae, apice integrae, 3–4 mm. longae, in sicco specimine subalbae. Paleae lineares, margine diaphanae. Achaenia attenuato-linearia, supra sparsim hispida, biaristata aristis glabris aut retrorsum 1–3-hamosis, (interiora) demum 0.8–1 cm. longa.

Ed. André 2878, at altitude of 1250 m., Rio Juanambu, United States of Colombia, April 28, 1876 (type in Herb. Gray).

The 20–30 areolae on the old disks become prominent as the involucres reflex. There are 37 heads on the type specimen. The plant appears to be entirely herbaceous, but the label says "suffrutesc.," hence the lower part of the plant (not present on the sheet) may have been woody and perennial.

Bidens mirabilis, sp. nov.[1]—Herba volubilis glabra, caule subtereto, striato. Folia caulis petiolo adjecto 8–10 cm. longa, pinnata, foliolis irregulariter 3- vel 5-partitis, segmentis (ad marginem sparsim hispidis) ovatis et integris aut ad apicem 1–5 dentatis, petiolis 3–3.5 cm. longis; folia ramulorum parva, integra aut ternata. Capitula (floribus flavescentibus e Sprucei inscriptione) multa, parva, dense paniculata, discoidea, 3–5 mm. lata, 4–5 mm. alta. Involucrum glabratum, squamis duplici serie dispositis; exterioribus lineare-lanceolatis, laciniato-ciliatis, 1.5–2.5 mm. longis. Paleae lineares, 4–5 mm. longae, marginibus diaphanis. Stilorum rami breves, crassi, plus minusve obtusi. Achaenia late cuneato-lanceolata, plana, margine lobulata, lobulis coronatis 1–4 longis pilis; constricta ad apicem cervice crassa, 8–10-aristata aristis inaequalibus et retrorsum hamosis.

Spruce 6273, Huambalpa, Andes Mountains, Peru ("Andes Quitenses, loco Huambalo"), November 1857 (type in Herb. Kew). Plate XXXI.

This is a very strange species and one that might be taken by some to represent a new genus; indeed, I know of no other species of *Bidens* combining

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[1] Dr. J. M. GREENMAN (now of the Missouri Botanical Garden), to whom I shall express more extended thanks later for having inspired my research upon the genus *Bidens* and directed it during its early stages, and Dr. B. L. ROBINSON (of Gray Herbarium) have very kindly assisted me with certain library and herbarium data for this species.

more diverse characters. The twining habit is to be met with in *B. rubifolia* and its allies; the large number of achene awns (very rarely so numerous in *Bidens*) suggests a certain African species of *Bidens*, to be treated later; the shape of the achenes is rather that of *B. tripartita* and its allies; but the thick, necklike constriction at the apex of the achenes and below the awns is so pronounced as to suggest a slight affiliation with *Heterospermum*,[2] in which genus the outer, *winged* achenes are frequently so constricted.

BIDENS PILOSA L. Sp. Plant. 832. 1753. *Bidens hispida* H.B.K. Nov. Gen. **4**:237. 1820; *Bidens andicola* H.B.K. var. *B*, Wedd. Chlor. And. **1**:70. 1855.

For many years, *Bidens hispida* H.B.K. appears to have remained a comparatively unknown form to botanists dealing with South American plants. WEDDELL's treatment of it is short and arbitrary. No reasons are given by him for its reduction to *Bidens andicola* H.B.K.

The original type plant of *Bidens hispida*, as also that of *Bidens andicola*, remains in good preservation today (in Mus. Hist. Nat. Paris). In a very general way the two plants, both of them hispid, show a resemblance; but *Bidens hispida* is seen, on closer inspection, to have small heads and these, moreover, are discoid. Also, the lower part of the plant displays a wholly different foliage aspect from that found in more recent specimens of the yellow-rayed *B. andicola* (the lower part of the type for the latter species is lacking). The plant is seen to be merely a very hispid form of *B. pilosa* L. It is matched more or less exactly by many specimens in herbaria, particularly by some from Venezuela, Colombia, and Peru (for example, *Mr.* and *Mrs. J. N. Rose* 18740, Santa Clara, Peru, July 18, 1914, in U.S. Nat. Herb.).

BIDENS ANTHRISCOIDES DC. Prodr. **5**:600. 1836.

DECANDOLLE's description of this species, based upon *Berlandier* 1010 and 1152, is much too narrow not to mislead. Excellent specimens of *Berlandier* 1010 are in London (in Herb. Brit. Mus.), and these have some achenes 21 mm. long. *Berlandier* 1010 and 1152 both occur in Paris (in Herb. Mus. Hist. Nat. Paris and in Herb. E. Drake). These surpass the London specimens in that they show some of the heads to be radiate in anthesis (cf. DC. *l.c.*, "cephalis discoideis"). The flowering heads are small, about 9 mm. high and 4–5 mm. wide, or, including the rays, 1 cm. wide. The rays are small, strongly and definitely dark-roseate, about 5 mm. long (including the tube at base), broadly ovate-elliptic, somewhat 3-toothed at apex. The 8 outer bracts average about three-fifths of the length of the inner ones and tend to be indurate-tipped.

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[2] A genus very closely connected with *Bidens* through *Heterospermum Xanti* Gray, the type of which (in Herb. Gray) is matched by that of *Bidens Xantiana* Rose (in U.S. Nat. Herb.); a species best retained in *Heterospermum*, a view in accord with the treatment by BRANDEGEE (Zoe 1:309. 1890), who, moreover, has since *positively* corroborated (in litt. 1913) my equation of *Bidens Xantiana* Rose with *Heterospermum Xanti* Gray.

BIDENS NODIFLORA L. Sp. Plant. 832. 1753.—*Bidens nodiflora, brunellae folio* Dillen. Elth. 52. *pl. 44. fig. 52.* 1732: non *Bidens nodiflora, folio tetrahit, ibid.* 53. *pl. 45. fig. 53.*

A good specimen preserved in the Linnaean Herbarium matches the first figure of DILLENIUS very closely and is a true *Bidens*. Recently, DRUCE (The Dillen. Herb. 161. 1907) has cited this figure erroneously, as representing, along with *pl. 45. fig. 53* (Dillen. *l.c.*), *Synedrella nodiflora* (L.) Gaertn., a species treated by LINNAEUS (Amoen. Acad. **4**:290. 1759) as *Verbesina nodiflora*, and which is not at all a *Bidens*. DRUCE, as is indicated by his use of an asterisk, had not seen a Dillenian specimen matching *pl. 44. fig. 52*, but he referred to the *Index Kewensis* as his authority. However, an examination of that work (Ind. Kew. **1**:301 and **2**:1025. 1895), with proper regard for the kinds of type there employed, shows that *Bidens nodiflora* and *Synedrella nodiflora* are retained as distinct species and are not equated.

BIDENS FRONDOSA L. Sp. Plant. 832. 1753. *Bidens melanocarpa* Wieg. Bull. Torr. Bot. Club **26**:405. 1899.

GREENE (Pittonia **4**:246. 1901) has given a good survey of pre-Linnaean authors and their treatment of the plant finally named *Bidens frondosa* by LINNAEUS. As additional evidence of the accuracy of GREENE'S conclusions regarding the characters of true *B. frondosa* L., there are the three specimens of LINNAEUS (two in Herb. Linn. and one in Herb. Hort. Cliff. at Herb. Brit. Mus.) and one of VAILLANT (in Herb. Mus. Hist. Nat. Paris). The first one of these has a flowering head with 9 or 10 elongate, foliose, exterior involucral bracts (cf. Linn. *l.c.*, "calycibus frondosis") and "*HU* 4. *frondosa*" is written on the sheet.[3] Pinned with this sheet is a second sheet having a plant without label, but which is coarser and has about 14 exterior involucral bracts on the largest head. The third specimen is among the *Hortus Cliffortianus* specimens and matches the first specimen, even to having the same elongate foliose type of exterior bracts. LINNAEUS clearly had the first or the third specimen, and probably both, in mind when he drew up his description of *B. frondosa* for the *Species Plantarum*. The second specimen is probably *B. vulgata* Greene, but it is not labeled, and has no historical significance. The fourth specimen is the one formerly in VAILLANT's private herbarium. This last matches the two labeled Linnaean specimens perfectly. Bearing, as it does, in VAILLANT's own handwriting, the early names[4] afterward cited by LINNAEUS as synonyms for *B. frondosa*, it shows that VAILLANT, himself a student of the genus *Bidens*, likewise understood this species to be the smaller headed, fewer bracted, less robust form (and not the *B. vulgata* of GREENE).

BIDENS CHINENSIS Willd. Sp. Plant. **3**:1719. 1800. *Bidens pilosa* L. var. *B* Murray, Syst. Veg. ed. 13. 610. 1774; *Agrimonia molucca*, Rumph. amb. **6**:38. *pl. 15. fig. 2.* 1750; *Chrysanthemum chinense*, etc., Plukenet, Phytograph. *pl. 22. fig. 4.* 1691; *idem* Almag. Bot. 100 (excl. syn.). 1696; *Bidens cicutaefolia* Tausch, Flora **19**:395. 1836.

Recently, O. E. SCHULZ (Engl. Bot. Jahrb. **50**: Suppl. 176. 1914) has presented a good historical summary of this species with a comprehensive mass of synonomy. He shows clearly that the name *chinensis* harmonizes in its application with the *chinense* of PLUKENET given over a century before. Evidently, however, he was unaware that the plate of PLUKENET's *Phytographia* (*loc. cit.*) was the identical plate cited in 1836 by TAUSCH (*Flora, loc. cit.*) as the basis for *Bidens cicutaefolia;* and that thus one of TAUSCH's ill-advised names, so long apparently enigmatic to botanists, is clearly reducible to synonomy.

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[3] Dr. B. DAYDON JACKSON, of the Linnaean Herbarium, assures me that "HU" was used by LINNAEUS to indicate that the plant was raised "*in Horto Upsalensi*."

[4] I am indebted to Professor P. DANGUY (of the Herb. Mus. Hist. Nat. Paris) for comparisons made with VAILLANT's known writing to verify the authenticity of these names. An extra label on the sheet "*Bidens frondosa* L." was written, according to Professor DANGUY, by LAMARCK.

Bidens humilis H.B.K. Nov. Gen. **4**:234. 1820.—*Bidens decomposita hirsutior* C. B. Clarke, Compos. Ind. 141. 1876.

Clarke described his variety *hirsutior* from a single specimen collected by himself at an altitude of over 2200 m. in the Nilgiri Mountains of India. Later, he informed J. D. Hooker (cf. Fl. Brit. Ind. **3**:310. 1881) that he supposed it to be some cultivated plant. Hooker admits having seen no specimen of it, but it happens that Clarke's own original specimen ("11207 . . . . 23 March 1870 . . . . coll. *C. B. Clarke* . . . .") was sent to Kew Herbarium in 1877 and is still there in good condition. The plant is very different from *Bidens decomposita* Wall., but differs from certain South American specimens of the highly variable *B. humilis* H.B.K. only in being rather villous. Yet in the same region Dr. Watt collected material (*Watt* 2160, Metapollium, Nilgiri Hills, Southern India, up to nearly 1000 m., June 1876, in Herb. Kew) that agrees with Clarke's specimen except that it is minutely pubescent as to leaves and glabrous as to stems. And, most fortunately, still two more specimens from this region occur (*Dr. Thomson*, Eclipse Exped., Nilgiris, December 1871, determined on sheet as "*Bidens humilis* H.B.K.," in Herb. Kew; and *R. H. Beddome* 4511, "introd. ? a common weed," Nilgiris, in Herb. Brit. Mus.), both glabrous and indistinguishable from *B. humilis*. A study of these several specimens, all collected in the same region at about the same time, and by two of the collectors suspected of being introduced, shows beyond doubt that they were merely forms of *B. humilis* H.B.K. brought, perhaps in ballast, from South America to the southwest shores of British India.[5]

Bidens crithmifolia H.B.K. Nov. Gen. **4**:234. 1820.—*Bidens delphinifolia* H.B.K. *loc. cit.*

The two type specimens from which Kunth described *B. crithmifolia* and *B. delphinifolia* are still extant in good condition (in Herb. Mus. Hist. Nat. Paris). They differ only in the slightly diverse foliage. The immature type of *B. crithmifolia* is matched exactly by *J. Triana* 1374 (*ibid.*), a specimen from Bogota, Colombia, which is superior in showing not only flowering heads but also numerous achenes. These achenes are mainly 2-aristate, but some are 3-aristate and so agree perfectly with achenes of *B. delphinifolia*.[6] Fortunately, I have found another specimen by *Triana* of the same number in the British Museum, and this shows the slightly different foliage of *B. delphinifolia*, thus removing all doubt as to the identity of *B. delphinifolia* with *B. crithmifolia*.

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[5] Since the above was written, I have had access to the very recent work of Fyson (Fl. Nilgiri and Pulney Hill-tops above 6500 feet 1:237. 1915). It is most interesting to find that Fyson, though omitting historical details, lists *B. humilis* from South America as an introduction into the Nilgiri Hill region, thus adding unique corroboration to my own conclusions presented above.

This species is exceedingly variable in foliage. *Triana* 1375 (in Herb. Brit. Mus.) shows one plant with leaves tripartite, the leaflets being incisely dentate, and another plant with simple, ovate-lanceolate leaves. In fact, the sheet of *Triana* 1374 in Paris bears another specimen (beside that cited above) which has similarly simple, ovate-lanceolate leaves, showing that in the field TRIANA considered the simple leaves and the finely divided leaves as belonging to the same species. More recently, further material has come from Colombia (*Herbert H. Smith* 1980, San Lorenzo ridges, Santa Marta, in Herb. Field Mus., Herb. New York Bot. Gard., etc.) which shows many leaves like those of the type of *B. crithmifolia*, but with leaf divisions narrower. A range of leaf outlines thus is shown that seems unbelievable, easily entitling *B. crithmifolia* to rank in this respect with such species as *B. heterophylla* Ort.

BIDENS HIRTELLA H.B.K. Nov. Gen. **4**:232. 1820.—*Bidens procumbens* H.B.K. *loc. cit.*

The type of *B. procumbens* (in Herb. Mus. Hist. Nat. Paris) differs only in the slightest way from that of *B. hirtella* (*ibid.*). KUNTH (H.B.K. *loc. cit.*) admitted the two forms to be very close. Had he possessed the wide range of data concerning variations in the related species of *Bidens* that, during the century since then, have steadily accumulated, he would not have hesitated to treat these as specifically the same.

Bidens chrysanthemifolia (H.B.K.). comb. nov.—*Cosmos chrysanthemifolia* H.B.K. Nov. Gen. **4**:239. 1820; *Cosmea chrysanthemifolia* Sprengel, Syst. Veg. ed. 16$^{\text{III}}$. 615. 1826; *Cosmos*

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[6] It is interesting to note that the mature head on the type of *B. delphinifolia* has at least one achene that is positively 2 aristate, showing no indication of a third awn ever having been present (cf. H.B.K. *loc. cit.*, "triaristata").

chrysanthemoides DC. Prodr. **5**:607. 1836; *Bidens Kunthii* Schz. Bip. Seem. Bot. Voy. Herald 308. 1852–1857; *Bidens parvulifolia* E. E. Sherff, BOT. GAZ. **56**:490. 1913.

This species was stated definitely by DECANDOLLE (*loc. cit.*) to come from Mexico, but KUNTH (H.B.K. *loc. cit.*) himself was uncertain as to its native country. DECANDOLLE, moreover, commented upon the closer affinity of the achenes with those of *Bidens* than with those of *Cosmos*. Whether he altered the specific name, however, through intent or through error, I am unable to say. But later, SCHULTZ BIPONTINUS (SEEM. *loc. cit.*), who frankly declared his belief that *Cosmos* was not a valid genus, used this altered name in citing it as a basis for his *Bidens Kunthii*, a name that, according to the Vienna Code, cannot stand.

ASA GRAY (Proc. Amer. Acad. **19**:16. 1884) strongly suspected that this plant was merely *Bidens humilis* H.B.K. and suggested a reexamination of the type material. KUNTH (H.B.K. *loc. cit.*) had described the color of the rays as "violacea, basim versus sulphurea." This description is borne out, not only by the coloring in the plate cited (that is, in copies of KUNTH'S work having the plates colored, as in John Crerar Library, Chicago), but by the type specimen in Paris (Herb. Mus. Hist. Nat. Paris), clearly the one from which the plate was made. This specimen, though discolored as to its rays, shows at least that the proximal ends of the rays were colored differently from the remaining portions, which latter seem surely to have been some shade of red.

More recently, fine material has been collected in Guatemala (*Heyde* and *Lux* 6173, alt. 900 m., Fraijanes, Dept. Amatitlan, September 1893, in Herb. Univ. of Chicago and in Herb. Kew), which belongs here. Singularly enough, it had been determined by JOHN DONNELL SMITH as *Bidens humilis* (cf. Gray *loc. cit.*), but the roseate rays and more or less *Cosmos*-like aspect are very distinct. The color of the rays in the dry condition varies from a pronounced roseate to a faded yellowish color, rather than showing a distinct sulphureous color definitely located toward the base as described by KUNTH. Still further material from the same small district in Guatemala (*W. A. Kellerman* 6112, alt. about 2500 m., Vol. Pacaya, Dept. Amatitlan, January 6, 1907, in Herb. Field Mus.) has been collected and fortunately is in a more mature condition. The mature achenes match the ovary figured by KUNTH and show that there is not the slightest tendency to become rostrate as in *Cosmos*.

An examination of the original description of *Bidens parvulifolia* (Sherff, *loc. cit.*) shows that the second cited specimen (which I had seen in the U.S. Nat. Herb.) was obtained by the same collectors at the same altitude, time, and locality as was *Heyde* and *Lux* 6173 (see foregoing). The dried ligules were yellowish, the leaves were pubescent, and all but the top pair were simple. Since then, however, I have found other specimens (*Heyde* and *Lux* 6162 and 6163) showing numerous intergradations between the simple, pubescent leaves and the compound, mainly glabrous leaves; also, variations to a roseate color are shown in the rays. Thus *Bidens parvulifolia*, incredible as it will seem to any botanist who does not have at hand the intermediate specimens, must be interpreted as merely a form of *Bidens chrysanthemifolia* having minute, mainly undivided leaves.

BIDENS CERNUA L. Sp. Plant. 832. 1753.[7]—*Bidens gracilenta* Greene, Pittonia **4**:255. 1901; *Bidens prionophylla* Greene, *loc. cit.* 256; *Bidens glaucescens* Greene, *loc. cit.* 258; *Bidens lonchophylla* Greene, *loc. cit.* 258; *Bidens Macounii* Greene, *loc. cit.* 259; *Bidens leptopoda* Greene, *loc. cit.* 260; *Bidens marginata* Greene, *loc. cit.* 262.

In 1901, GREENE (*loc. cit.*) described a number of new species of *Bidens*. On examination of his types (mostly in U.S. Nat. Herb.) and cotypes (mostly in Herb. Gray and in Herb. Field Mus.) nearly three years ago, I was dismayed to find that most of these species represented what ordinarily had been regarded as mere ecological forms of *Bidens cernua* and *B. laevis*. Direct conversation with Dr. GREENE himself showed that back of his viewpoint regarding *B. cernua* (Greene *loc. cit.* 251–253) was the absolute conviction that the American specimens were native to America, and, being so, were hence specifically different from European specimens.[8] However, personal field study for the past four autumns, combined with a careful examination of a vast amount of *B. cernua* material from different American and European stations, has convinced me only the more of the utter impossibility of separating the many forms as species. Scarcely a form occurs in the United States that is not duplicated by a precisely similar form in Europe. Even Greene himself (*loc. cit.* 252) was compelled to declare "after careful and repeated comparisons made between European and American specimens of so-called *B. cernua*, I acknowledge inability to detect any strong technical characters upon which to separate them."

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[7] Here, as elsewhere in this series of articles, only the most relevant synonyms are cited, the others being left for a complete monographic treatment later.

[8] In emphasizing his views upon this subject, Dr. GREENE exclaimed: "I defy you to find a single species of Compositae that is native both to Europe and to North America." It is not my intention to discuss this opinion here, since it is quoted merely to show his viewpoint. I do desire, however, to acknowledge with gratitude the courtesies shown me by Dr. GREENE from time to time. For over two years we had been making field observations for each other upon various species of *Bidens*, but his recent death brought this mutual aid to an abrupt end.

Again, in a single colony of *B. cernua*, frequently three or more dissimilar forms occur, with numerous intergradations. Thus in a single small colony north of Elgin, Illinois, many plants were diminutive, matching *B. minima* Huds.; some were tall and robust, matching *B. leptopoda* Greene (the type of which Professor J. M. Macoun of the Canadian Geol. Surv. Herb. at Ottawa kindly permitted me to examine); and some were small plants grown from the rooting nodes of tall plants trampled down by cattle, and were practically identical with *B. marginata* Greene. In the same way, several of Greene's types are found on comparison with their cotypes in other herbaria to be merely slight variants from the standard form. In several of these cases, Greene's description was much too narrow to fit even the few cotypes examined.

Bidens amplissima Greene, Pittonia **4**:268. 1901.

This species has been discussed already (Sherff, Bot. Gaz. **59**: 312. 1915). The name *Bidens elata* then proposed as a substitute has since been found, however, at variance with an example cited in the Vienna Code for a similar case. Hence *B. elata* cannot be retained as technically the valid name.

Bidens laevis (L.) B.S.P. Prelim. Cat. N.Y. 29. 1888.—*Helianthus laevis* L. Sp. Plant. 906. 1753; *Helianthus foliis lanceolatis serratis laevibus* Gronov. Fl. Virg. **1**:104. 1739;[9] *Bidens chrysanthemoides* Michx. Fl. Bor. Amer. **2**:136. 1803; *Bidens helianthoides* H.B.K. Nov. Gen. **4**:230. 1820; *Bidens elegans* Greene,

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[9] I could not find Clayton's no. 195 among the Gronovian plants at the British Museum, and am unable personally to confirm this citation except from Gronovius' description. But, in 1860, Asa Gray (so Miss Mary A. Day of Gray Herbarium has kindly ascertained for me) worked upon the Gronovian plants of the British Museum and listed Clayton's no. 195 as "195 *Bidens chrysanthemoides!* (not *Heliopsis laevis*)."

Pittonia **4**:254. 1901; *Bidens lugens* Greene, *loc. cit.*; *Bidens formosa* Greene, *loc. cit.* 264; *Bidens Parryi* Greene, *loc. cit.* 265; *Bidens persicae-folia* Greene, *loc. cit.* 266.

The types of *Bidens chrysanthemoides* Michx. and *B. helianthoides* H.B.K. (both in Herb. Mus. Hist. Nat. Paris) appear precisely the same. The original description of both species shows their achenes to have been 2-aristate in each case, although many specimens have since been gathered showing the achenes often 3 or 4-aristate. A study of numerous specimens from the United States and Mexico seems to indicate a slight tendency for the western specimens to be more often 2-aristate, the eastern ones more often 3 or 4-aristate. But the variations are so abundant as to defy all attempts at delimiting the separate forms or races in a specific way (cf. TORR. and GR. Fl. N. Amer. **2**:353. 1842).

GREENE cites a single sheet for *B. formosa*, a plant from Delaware County, Pennsylvania. But in the Field Museum are 5 sheets of material (all by *J. K. Small*, Wetzel's Swamp, N. Harrisburg, September 1887) from the same state, and these show all gradations between *B. formosa* and *B. laevis*. Again, GREENE terms his *B. Parryi* an unwelcome species, "as uniting the habit of *B. cernua* and the fruit of the Platycarpaea group of species." But even if *B. Parryi* were a valid species, it would not be the first species to do this; for all the material of *B. laevis* that has flat, biaristate achenes does the same; and, moreover, *B. radiata* Thuill. (*B. platycephala* Oerst.) had long been noted as a species that likewise united *B. cernua* with *B. tripartita*, the latter a principal species of the Platycarpaea group (cf. G. SCHWEINFURTH, Verhand. Bot. Verein Prov. Brand. **2**:145. 1861). Indeed, GREENE himself, on another occasion (*loc. cit.* 261) had been led to consider *B. radiata* in this same connection, having suspected his *B. leptopoda* as being this species. DECANDOLLE, in monographing the genus *Bidens* (Prodr. **5**:594. 1836), defined the subgeneric section *Platycarpaea* with the evident purpose of admitting just such species as *B. cernua*, and actually classed *B. cernua* among the *Platycarpaea*.

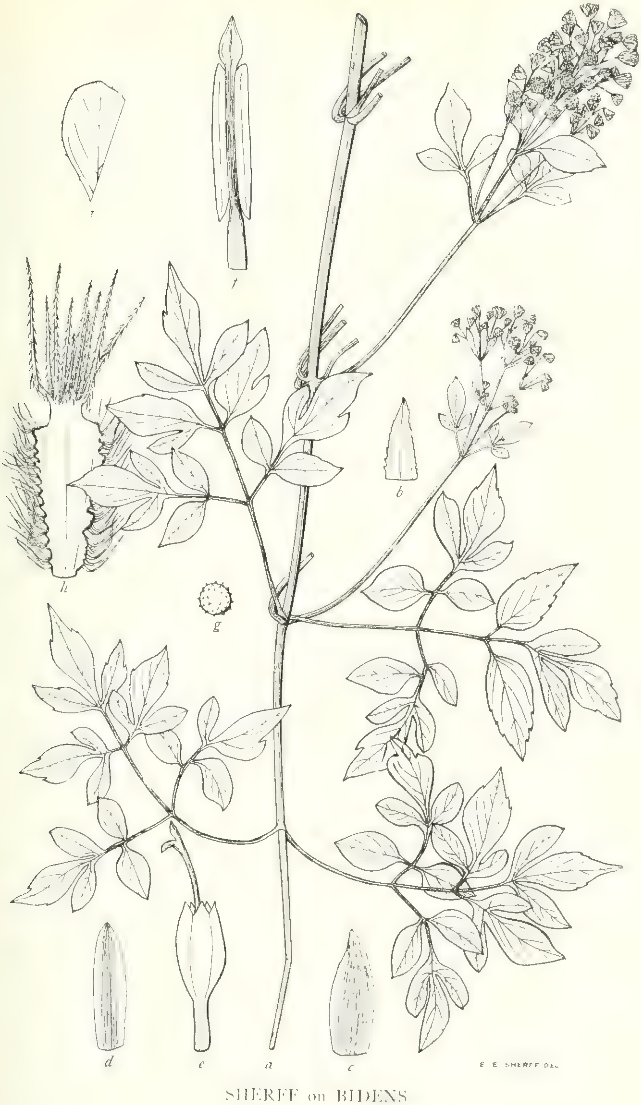
Hence GRAY saw this plant and determined it personally. As his knowledge of this species was very keen, there is no question as to his accuracy. LINNAEUS' private specimen of *Helianthus laevis* L. (in Herb. Linn.) is *Heliopsis* (cf. GRAY, Synopt. Fl. 1^{II}:255. 1884; also PERSOON, Synops. Plant. **2**:473. 1807).

Several other species described by Greene and based upon characters seemingly inconsistent or difficult at present to evaluate, are reserved for later treatment.

University of Chicago

EXPLANATION OF PLATE XXXI

Bidens mirabilis.—*a*, spray ×0.7; *b*, exterior bract ×4; *c*, interior bract ×4; *d*, palea ×4; *e*, floret ×7; *f*, anther ×35; *g*, pollen grain ×350; *h*, achene ×11; *i*, portion of leaflet ×3; all from type in Herb. Kew.



SHERFF on BIDENS

With regards of Chas A Shull

MEASUREMENT OF THE SURFACE FORCES IN SOILS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 217

(WITH FIVE FIGURES)

CHARLES ALBERT SHULL

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MEASUREMENT OF THE SURFACE FORCES IN SOILS

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CHARLES ALBERT SHULL

(WITH FIVE FIGURES)

I. Introduction

Many investigations of soil moisture have been made, especially during recent years, in attempts to learn something of its mechanics and its relations to plant growth. In a general way the dominating importance of the soil water to plants has long been recognized; but notwithstanding the large amount of work done up to the present time, we still lack some of the most fundamental, elementary facts regarding the physico-chemical relations of the water and soil. This is true generally of that region of soil moisture which lies between what is called the wilting coefficient of the soil and air-dry soil, and more particularly of that critical region immediately below the wilting coefficient.

The discovery of semipermeable coats in seeds in recent years by BROWN (4), SCHRÖDER (30), and others has made it possible to measure approximately the force with which the colloidal gels of the seed attract water. In a former paper (33) it was shown that by means of osmotic solutions whose forces are known the imbibition force of a seed at any given moisture content from saturation to air-dry could be determined approximately.

Because of the rapid establishment of moisture equilibrium relations after disturbance in *Xanthium* seeds, this seed has been

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chosen for a further investigation of the moisture relations of seeds, with special reference to the moisture held by soil particles. The main purpose of the work was to find some means of measuring the force with which particles of soils of varying fineness retain moisture at different degrees of dryness, and to obtain some more definite knowledge concerning the amount of "back pull" occurring in soils when the total moisture content is so low as to be unavailable to growing plants. Special interest centered in the conditions obtaining in the critical region at and just below the wilting coefficient.

This paper presents the principal results obtained during the last three years. Since the osmotic method of measuring the internal forces of seeds is obviously restricted in practice to such seeds as have a perfectly semipermeable coat, a new method was attempted, based upon a determination of the vapor pressure equilibrium between seeds and osmotic solutions of varying strengths. This method has the advantage of being applicable to all sorts of seeds, regardless of the kind of testa present; but since VON SCHRÖDER (**31**) and BANCROFT (**2**) have shown that colloids may not have the same moisture relations to gaseous moisture that they have to water itself, the values obtained by the vapor pressure method have not been used as the basis of comparison with soils in this work. The values for the internal force of seeds as determined by osmotic solutions of various strengths will therefore be used as a basis for comparing the moisture-holding power of fine soil particles.

A number of soils have been used in the investigations, and it is believed that the methods of measurement used here will prove valuable in many kinds of soil moisture studies, since the determinations, while giving excellent data as to the physical relations of the soil moisture, yield at the same time results of considerable physiological significance. The results are more valuable, therefore, than purely physical determinations, because they can be interpreted in terms of plant activity. For, after all, it is the plant in relation to its environment, not merely the environment, that we need to understand.

The work has been carried on in the Plant Physiological Laboratory of the University of Kansas, and in the Hull Botanical

Laboratory of the University of Chicago, where all needed facilities have been generously provided.

II. Historical

The general status of our knowledge of the forces operative in soils was briefly discussed by CAMERON (**10**) several years ago. It is obvious from this account that up to the present time we have known very little about soil forces within the range of unavailable moisture, that is, between the wilting coefficient and air-dry condition of the soil.

The attempts thus far made at measurement of the surface forces which are known to exist in finely divided matter of all kinds have been made from various angles, but they can be classed under two main heads: (*a*) physical, and (*b*) physiological.

PHYSICAL MEASUREMENTS

A. HEAT OF WETTING METHOD.—The principle of heat of wetting was discovered by POUILLET (**26**) a good many years ago. He found that all kinds of dry powders, from inorganic substances and porous organic matter, yielded heat on being wet with fluids like water, oil, alcohol, etc. The organic substances yielded the greater amount of heat because, he stated, the organic matter was composed of particles incomparably thinner than the finest inorganic powders.

The literature dealing with the application of this principle to measurements of surface force has been reviewed so recently by PATTEN (**25**) that it will not be necessary to go into the details of it here. It will be sufficient to point out that through the work of ROSE (**28**) and JUNGK (**19**) we gained the conception that water is condensed on the surface of the powdered inorganic or finely divided organic substances, and that the release of heat is due to this compression. The quantitative studies of NÄGELI (**23**) made it possible for SACHS (**29**) to calculate the surface forces in starch grains. Since JOULE had shown that 34.3 atmospheres of pressure raises the temperature of water 0.03° C., the amount of heat produced by starch on being wet would indicate much more than 10,000 atmospheres of surface force compression. SACHS assumed, of course, NÄGELI'S theory of the structure of organic matter.

The physicists YOUNG, DUPRË, and Lord RAYLEIGH have estimated the surface forces of finely divided absolutely dry matter at from 11,000 to 25,000 atmospheres. LAGERGREN'S (**20**) estimate for charcoal fine enough to have 4 sq. m. of internal surface per gram was 6150 atmospheres.

It is probable that this method would give results too high for soils, for, as MÜNTZ and GAUDECHON (**22**) have shown, there are other sources of heat release than mere compression when absolutely dry soil and water are mixed. Heat of solution, dilution, and hydration may make considerable errors in estimates of surface forces by this means. The statement frequently made that the force of surface condensation in soils runs from 6,000 to 25,000 atmospheres, as by CAMERON (**10**), and by BROWN and SMITH (**5**), is based upon the discussion previously mentioned.

B. COMPRESSION METHOD.—RODEWALD (**27**) has used a different method in measuring the forces on the surface of starch particles, which has the advantage of being a direct method; that is, the forces of compression are measured by the amount of compression produced instead of by the amount of heat produced. He found that 1 gm. of oven-dry starch absorbed 0.326 gm. of water in becoming saturated. But while the starch swelled, the swelling did not amount to as much as the volume of water absorbed. In other words, there was a volume loss due to compression of the water. The amount of volume loss was 0.0432 cc., and if we refer this to a gram of water, the volume loss is equal to 0.1325 cc. per gm. The compression coefficient of water is calculated by WÜLLNER to be 0.00004659 cc. per gm. for each atmosphere of pressure exerted. This would give a pressure of 2821 atmospheres for the compression actually obtained if we refer the compression solely to the water involved.

By a slightly different method of calculating the force of compression RODEWALD obtained a result of 2523 atmospheres, which is not referred to the water alone, but to the whole system of starch and water. He thinks that the close agreement shows that water alone is involved, or that starch happens to have about the same coefficient of compressibility as water.

The low value obtained by RODEWALD as compared with the values for inorganic bodies, PATTEN thinks is due to the fact that imbibition and absorption are both involved in the starch, and that a much lower value must be obtained than where absorption alone occurs. On the other hand, it will give a higher value than where imbibition alone occurs.

While these determinations of the surface force in absolutely dry matter are interesting, they have no practical value, for such forces as these do not occur in ordinary soils containing capillary moisture, or even in air-dry soils and seeds, for it is evident that the air-dry soil or seed already holds as hygroscopic moisture the water that it would absorb with such remarkable energy if the particles were absolutely dry. However, the figures give us an idea of the power with which these substances retain the last part of their hygroscopic moisture, which must be a force opposite to and equal to that with which wetting occurs.

C. VAPOR PRESSURE AND CENTRIFUGAL FORCE METHODS.—Other physical measurements have been worked out, some of which are very useful, as for instance HILGARD's hygroscopic coefficient (**16**), a measure based on vapor pressure relations, and the moisture equivalent of BRIGGS and McLANE (**6**). The latter is particularly valuable, since BRIGGS and SHANTZ (**7**) have shown its relation to various physical and physiological amounts of water. But only one of these measurements can be expressed at present in units which permit a comparison of the soil forces with the osmotic forces of the roots of plants.

PHYSIOLOGICAL MEASUREMENTS

The most important attempt at a physiological measurement of the soil forces is that of BRIGGS and SHANTZ (**8**), who use the wilting coefficient, or percentage of moisture in the soil at the wilting of the plant, in determining unavailable moisture. However, recent work by CALDWELL (**9**) and by SHIVE and LIVINGSTON (**32**) shows that within certain ranges the permanent wilting of the plant is a function of the intensity of atmospheric evaporation, and that the wilting coefficient should be rather a measure of the moisture in the plant at the time of wilting than of the moisture in the soil. The constancy of this measure is therefore open to some question, and its value and limitations in physiological studies are to be determined.

Another important physiological study of soil-moisture relations is ALWAY'S (1) investigation of the relation of non-available water to the hygroscopic coefficient. He has shown that some kinds of plants can remain alive for a considerable time after growth ceases from lack of moisture, while others die rather promptly. This is doubtless one of the main differences between xerophytes and mesophytes. In the case of desert perennial legumes, life was maintained even after the soil moisture had fallen slightly below the hygroscopic coefficient. These results emphasize the need of a measure for the surface force of soils which can be expressed, or at least interpreted, in biological rather than physical terms.

There have been few observations on the relation of seeds to soil moisture. BOGDANOFF (3) studied the relation of germinating seeds to soil moisture, and presents many interesting facts. WHITNEY and CAMERON (36) noted the fact that a quantity of cowpeas whose hygroscopic moisture amounted to about 14 per cent, when mixed with an equal quantity of soil which contained 15 per cent of water, took up 12.1 per cent of their own weight, leaving only 1.3 per cent of moisture in the soil. That is, the soil was practically air-dry. In the paper referred to (33) I have shown that the initial internal force of air-dry seeds is little short of 1000 atmospheres; if this condition be general among air-dry seeds, the behavior of the cowpeas can easily be understood. The relation of seeds to soil moisture and vapor pressure will be considered in more detail later.

III. Materials and methods

MATERIAL. The *Xanthium* seeds used in the experiments discussed in the following section were secured from plants raised on the experimental grounds of the University of Kansas in 1913. Originally all of the seeds planted were from a single plant of *X. pennsylvanicum* Wallr. The 119 plants obtained were very uniform in all their obvious characters, and since it has been shown (34) that the intermingled local types of *Xanthium* are practically isolated by differences in the blooming time of each species, the seeds may be considered as having come from a fairly pure line. This was thought desirable in order that the individual variations of the seeds might be reduced to a minimum, and that consequently more uniform behavior might be obtained under experimental conditions. The other seeds used were obtained from local seedsmen under the names given.

The soils used in the major portion of the work will be characterized briefly. As a representative of heavy clay soil, the subsoil of the Oswego silt loam was chosen. Specimens of this subsoil were obtained from Riley County, Kansas, on an area about 2 miles west of Manhattan. The Oswego silt loam is a residual soil derived by weathering from underlying unbedded shales and sandstone, with the shales predominating. Its subsoil forms a hard, compact, brittle soil, with a gray to dark brown color. The average composition as determined by mechanical analysis is given in table I.

TABLE I

Sand				Silt	Clay
Coarse	Medium	Fine	Very Fine		
0.4 per cent	0.5 per cent	4.4 per cent	3.2 per cent	61.3 per cent	30.4 per cent

The moisture equivalent is 35.2 per cent, and the wilting coefficient is 19.1 per cent. The general details in regard to the Oswego silt loam and its subsoil may be obtained from the Eighth Report, Field Operations of the Bureau of Soils (**11**).

As a contrast to the heavy silt clay, a fine quartz sand, the no. 2.0, which is manufactured by the Wausau Quartz Company from quartz rock, was chosen. This grade passes through a 124-mesh screen, and over a 147-mesh screen. The average diameter of the particles is very close to 0.10 mm. The chemical analysis given below shows it to be a very pure quartz sand.

Silicon dioxide	99.07 per cent
Iron oxide	0.17
Aluminum oxide	0.52
Hygroscopic moisture	0.06
Undetermined	0.18
	100.00

The moisture equivalent is 2.41 per cent and the wilting coefficient 1.3 per cent.

For a comparative study of the moisture relations of seeds and soils at the wilting coefficient of the soil, a series of soil samples was obtained from Washington, D.C. The necessary data regarding these soils are given in table II.

TABLE II

Sample number	Locality	Soil type	Moisture equivalent	Wilting coefficient	Hygroscopic H_2O (per cent)*
1	Yuma, Arizona	Sand	1.35±0.04	0.73±0.02	0.205
2	Highmore, South Dakota	Loam	23.79±0.10	12.93±0.05	3.13
3	North Platte, Nebraska	Very fine sandy loam	15.33±0.08	8.33±0.08	1.836
4	North Platte, Nebraska	Loam	22.84±0.03	12.41±0.02	2.28
5	Amarillo, Texas	Clay loam	29.65±0.02	16.12±0.01	3.82
6	North Platte, Neb.	Clay loam	30.08±0.04	16.34±0.02	5.21
7	Akron, Colorado	Fine sand	5.90±0.05	3.21±0.03	0.75
8	Yuma, Arizona	Sand	1.53±0.01	0.83±0.01	0.218
9	Akron, Colorado	Loam	19.91±0.12	10.82±0.06	2.3

* The hygroscopic moisture was determined at the time of use in an ordinary dry oven. The other figures were furnished by Dr. LYMAN J. BRIGGS, of Washington, D.C.

METHODS.—While the internal forces of *Xanthium* seeds have been approximated by osmotic means, many seeds lack semipermeable coats. For such seeds a vapor pressure method has been used which gives results which are in a way comparable to the osmotic measurements. It consists essentially in measuring the vapor pressure equilibrium of the air-dry seeds over sulphuric acid of varying strength, and calculating the internal pressure of the seed from the vapor pressure of the solution over which it was found to be in equilibrium. Owing to our slight knowledge of the concentrated solutions and of the exact relations of colloids to water vapor, the calculations can give only a rough estimate of the internal forces of the seeds, but they are near enough to the osmotic determinations to be of great interest.

The sulphuric acid series was chosen with some reference to the LANDOLT-BÖRNSTEIN tables to facilitate calculation. Beginning with water, the series included 16, 26.5, 35, 39, 50, 54, 57.5, 66, 73, 84.5, and 96–99 per cent H_2SO_4. These fluids were placed in tightly sealed, small, wide-mouthed bottles. The seeds to be tested were suspended in shallow paper baskets a few millimeters above the surface of the acid, the baskets being hung on cotton threads fastened to the corks with carna-uba wax. All metallic condensers were thus avoided. After the seeds were carefully weighed and arranged, the bottles were sunk in a trough of running water to prevent any considerable changes in temperature. Condensation effects due to change of temperature could not occur except over water, for MITSCHERLICH (**21**) has shown that even 10 per cent sulphuric acid will prevent deposition of dew in determining hygroscopic coefficients of soils. It may be questioned whether the inclosed space actually reaches the vapor pressure of the solution, for, as HILGARD (**17**) points out, it is most difficult to secure complete saturation in the case of water vapor. However, the space of air to be brought into equilibrium with the solution vapor pressure in these experiments is very small, and it seems probable that the whole system of liquid, air, and seed comes to an equilibrium pressure in the time of the experiment, except possibly in the case of water. After allowing 15 days for reestablishment of equilibrium by the seeds, the point of no change was determined by weighing.

The osmotic pressure of the sulphuric acid is roughly determined by the use of the vapor pressure formula $P=\frac{f-f^1}{f}\cdot\frac{SRT}{M}$, in which f is the vapor pressure of pure water at the temperature of the experiment, f^1 the vapor pressure of the acid, M the molecular weight of the solvent's vapor, T the absolute temperature, S the density of the sulphuric acid, and R the gas constant. The osmotic pressure (P) is given in grams per square centimeter, and must be reduced to atmospheres. This formula has been developed for dilute solutions and does not hold accurately for high concentrations, but there are at present no data on which to base more accurate estimations. The boiling-point method yields a result close to that given by this formula for sulphuric acid, as will be shown later.

The earliest soil measurements were made with no. 2/0 sand. Seeds of known weight were packed firmly in sand of known water content in paraffined wire baskets, and allowed to come to equilibrium. The tests were confined finally to the region of soil moisture from air-dry to the wilting coefficient, for with a higher moisture content the seeds always became saturated with water. In the case of this sand it was not until the water content was reduced to about 1 per cent that a noticeable "back pull" was developed by the soil.

This method is obviously open to the criticism that friction retards the movement of water in dry soils, and that the seeds therefore do not reach actual equilibrium with the total soil mass, but only with the soil lying very near them. In order to meet this difficulty a rotation method was adopted which brings the seeds constantly into contact with fresh soil particles.

A definite amount of dry soil, usually 60 gm., was taken, and the desired amount of water thoroughly mixed with it. In this condition the soil was divided finely enough to pass through a 2 mm. sieve. The moist soil was then placed with dry seeds in a wide-mouthed 200 cc. bottle, without completely filling it, so that rotation would constantly mix the soils and seeds. The bottles were carefully sealed with heavily shellacked corks to prevent loss of water during the period of rotation.

These bottles were arranged on rotating wheels, driven by a motor and controlled by a speed reducer (fig. 1). The range from air-dry to wilting coefficient was divided into 10 fairly equal divisions, giving 11 tests in each series. The rotation was continued for 15 days, this time having been chosen after making tests as to the effect of differences in duration of the experiment on the amount of intake by the seeds. For instance, no. 2/0 sand with about 0.2 per cent moisture added permitted an intake of 22 per cent of their weight by the seeds in 5 days, and a parallel test showed 21.7 per cent intake in 10 days. Fifteen days, therefore, seems ample time for the establishment of equilibrium. At the end of the time the bottles were opened, and the seeds very rapidly separated from the soil and brushed free of all dust with a camel's-hair brush. The soil and seeds were both placed in weighing bottles as quickly as possible, to prevent serious loss of water by evaporation. The soil was weighed carefully and dried at 104° C. until loss ceased. The seeds also were weighed. The moisture content of the seeds and soils at the time the bottles were opened expressed the equilibrium relation of that soil moisture content.

Since means are at hand for determining the internal force of the seed at practically any moisture content, it is possible to determine from the data the forces of the soils which are in equilibrium with those of the seed.

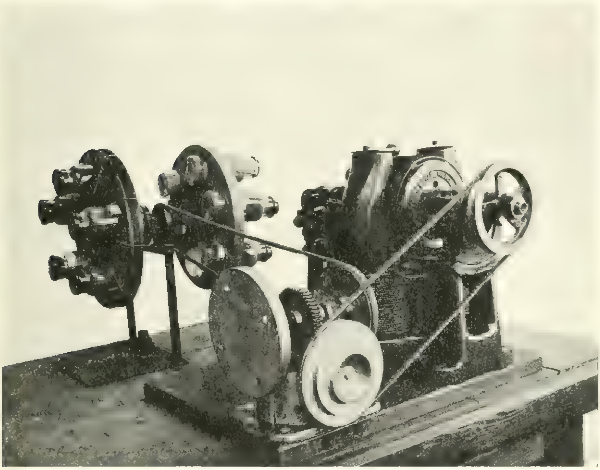

FIG. 1.—Rotator used in these experiments, with motor and speed reducer.

The principal sources of error lie in the fact that moist soils and seeds cannot be handled in ordinary atmospheres without some loss by evaporation during the handling, and in the fact that hot-air ovens for drying are not as accurate as vacuum driers. No claim is made for greater accuracy than these methods will permit. Of course, every precaution was taken to reduce errors to a minimum, and the work was done with the greatest speed and accuracy possible. It is confidently believed that the results to be obtained by more refined methods and more expensive apparatus would in no way change the nature of the conclusions to be drawn from the results.

IV. Experimental results

A. MEASUREMENT OF THE SEEDS.—The measurement of the internal forces of *Xanthium* seeds by means of NaCl and LiCl solutions has been repeated and extended with full confirmation of the previously published results. The data are presented in tabular form for the sake of convenience in table III, and these figures may serve as a basis of comparison in the soil experiments, where the surface forces of the soil particles, instead of osmotic pressure, are pitted against the internal forces of the seed. The data were secured with the lower seeds of *Xanthium pennsylvanicum*.

TABLE III

MOISTURE INTAKE OF *Xanthium* SEEDS IN OSMOTIC SOLUTIONS; TEMPERATURE 23.5° C.; INTAKE IN PERCENTAGE OF AIR-DRY WEIGHT

Solutions volume molecular	1 hour	4 hours	7 hours	10 hours	24 hours	48 hours	Osmotic pressure in atmospheres
H_2O............	16.39	44.38	48.78	50.38	51.18	51.58	0.0
0.1 M–NaCl....	16.79	39.43	45.87	46.48	46.39	46.33	3.8
0.2 M–NaCl....	17.12	38.67	45.00	45.57	45.93	45.52	7.6
0.3 M–NaCl....	16.07	34.05	40.75	41.95	42.24	42.05	11.4
0.4 M–NaCl....	14.36	31.21	38.08	39.97	40.33	40.27	15.2
0.5 M–NaCl....	13.96	30.26	35.87	38.08	38.70	38.98	19.0
0.6 M–NaCl....	13.80	25.57	32.41	33.57	34.77	35.18	22.8
0.7 M–NaCl....	13.32	26.29	30.99	31.73	32.79	32.85	26.6
0.8 M–NaCl....	13.13	25.22	29.21	29.95	31.12	31.12	30.4
0.9 M–NaCl....	12.58	24.34	27.64	28.95	29.14	29.79	34.2
1.0 M–NaCl....	11.90	22.92	25.42	26.48	26.21	26.73	38.0
2.0 M–NaCl....	8.19	14.55	18.25	18.43	18.60	18.55	72.0
4.0 M–NaCl....	4.81	8.37	9.84	10.08	11.00	11.76	130.0
Sat. –NaCl....	3.42	4.94	5.24	5.84	6.21	6.35	375.0
Sat. –LiCl.....	−0.67	−0.77	−0.58	−0.58	−0.58	−0.29	965.0

The results obtained by the vapor pressure method over sulphuric acid are shown in table IV, and some of the curves of moisture intake showing the point of natural equilibrium are shown in figs. 2 and 3.

As the table and curves show, the seeds are initially in moisture equilibrium with sulphuric acid of 46–54 per cent strength. In general the seeds which have carbohydrate reserves in greatest abundance seem to have a somewhat lower equilibrium point than those with high fat and protein content.

The osmotic pressure of the sulphuric acid calculated from the vapor pressure formula given runs from 1000 to 1350 atmospheres. The validity of the vapor pressure formulae will be discussed later. If colloids absorbed as much moisture from a saturated atmosphere as from water, it might be safe to assume that the internal force of the seeds is equal to the osmotic force of the solution. But if VON SCHRÖDER'S work holds for all colloids, this vapor pressure method may give abnormal values. If the colloids always tended to take up more water when in contact with the fluid, above the equilibrium point as well as below, the values given here would be too low, as the equilibrium point would be shifted toward the stronger acids. If, on the other hand, intake is increased below the equilibrium point, and loss is increased correspondingly above the equilibrium point, the shape of the curve would be changed, but the equilibrium point would remain fixed.

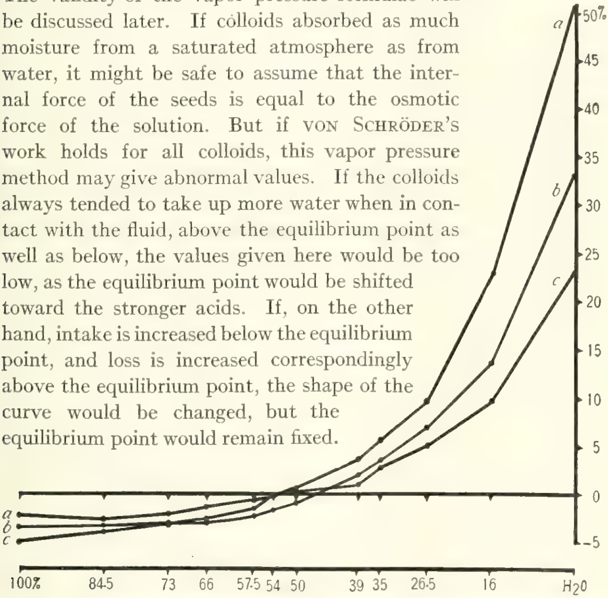

FIG. 2.—Curves of moisture equilibrium of seeds suspended over sulphuric acid: *a*, *Pisum sativum;* *b*, Stowell's evergreen sweet corn; *c*, *Xanthium pennsylvanicum;* abscissae, strength of sulphuric acid; ordinates, moisture intake by seeds in percentage of air-dry weight.

The values run higher with the H_2SO_4 than with the lithium chloride solution, as given in table III. There is one source of possible discrepancy which needs to be mentioned. The vapor pressure tests were all made in Kansas, where the climate averages drier than at Chicago, while the osmotic measurements were all

TABLE IV

VAPOR PRESSURE MEASUREMENT OF SEEDS; GAIN AND LOSS IN WEIGHT OF SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT

SEEDS	GAIN					GAIN AND LOSS	LOSS						EQUILIBRIUM POINT	HYGROSCOPIC MOISTURE	INTERNAL FORCE CALCULATED IN ATMOSPHERES
	Specific gravity H_2SO_4→	1.110	1.190	1.258	1.294	1.308	1.436	1.473	1.566	1.647	1.772	1.835			
	H_2O	Percentage H_2SO_4→ 16	26.5	35.0	39.0	50	54	57.5	66	73	84.5	96.99			
													Per Cent	Per Cent	
Oryza sativa.....	20.09	10.52	5.77	3.11	1.51	−0.81	1.78	2.62	3.92	4.71	4.97	5.29	46.15	10.12	1008.0
Oryza sativa....	18.1	12.40	7.70	3.50	1.70	−0.80	0.77	1.70	2.80	4.30	5.80	5.90	49.50	9.9	1182.0
Reid's yellow dent corn..........	18.99	10.97	6.89	3.31	2.16	−0.67	1.46	2.39	3.43	3.51	3.46	3.97	47.40	11.96	1060.0
Stowell's evergreen sweet corn	33.42	13.96	6.87	3.36	1.85	−0.89	1.68	2.26	3.07	3.08	3.33	3.42	46.40	11.63	1019.0
Feterita..........	27.00	11.96	7.02	3.62	2.88	+0.74	0.40	1.54	3.23	4.47	5.09	5.43	52.60	6.06	1284.0
Xanthium pennsylvanicum	22.9	9.86	5.09	2.61	0.98	+0.42	0.00	1.68	2.72	3.11	3.88	5.11	54.00	5.28	1354.0
Xanthium pennsylvanicum	30.95	10.11	4.46	2.86	1.36	+0.46	0.08	0.37	1.13	1.73	3.02	3.05	53.40	5.83	1324.0
Xanthium pennsylvanicum	20.63	15.86	9.45	5.85	3.01	+1.63	0.13	0.48	1.33	1.77	2.98	1.99	53.70	5.16	1340.0
Ricinus communis.....	18.1	6.67	2.75	1.47	1.20	+0.15	0.18	0.49	0.93	1.9	2.4	2.5	51.80	3.32	1246.0
Triticum sativum.	40.53	14.20	8.84	5.18	3.23	+0.78	0.00	0.64	2.54	3.24	3.70	3.44	54.00	10.19	1354.0
Pisum sativum (wrinkled seeds)	50.56	23.01	9.76	5.61	3.54	+0.60	0.26	0.56	1.33	2.15	2.57	2.19	52.80	8.14	1294.0
Soy beans.......	40.46	18.59	8.59	3.72	3.22	+0.70	0.00	0.62	1.50	2.90	3.12	3.16	54.00	6.88	1354.0
Lupinus albus....	24.06	5.58	4.13	2.59	2.05	+0.85	0.31	0.31	1.07	2.05	1.87	2.02	52.90	8.02	1300.0

made at Chicago. The hygroscopic moisture of the seeds was uniformly near 5.5 per cent in Kansas,[1] while at Chicago the hygroscopic moisture was rarely less than 7 per cent, usually above. The equilibrium point would naturally run higher, therefore, in a drier climate. However, it is not claimed that the discrepancy would entirely disappear if repeated with seeds of the same moisture content.

There seems to be little doubt as to the high osmotic strength of the sulphuric acid, for the boiling-point method of measuring osmotic pressure gives a value in close agreement with those just given. The boiling point of 53 per cent H_2SO_4 is 128.5° C. If for each rise of 0.52° C. we may assume an osmotic pressure equal to one mole of dissolved substance (Höber, Phys. Chem. p. 19), this strength of sulphuric acid should have an osmotic pressure of 1227 atmospheres.

At all events, it is safe to conclude that air-dry seeds possess a very high internal attraction for water which at the initial moment of intake is but little short of 1000 atmospheres. Owing to uncertainty of the figures for sulphuric acid, only those obtained by direct contact with osmotically active solutions will be used as a standard for the following soil experiments:

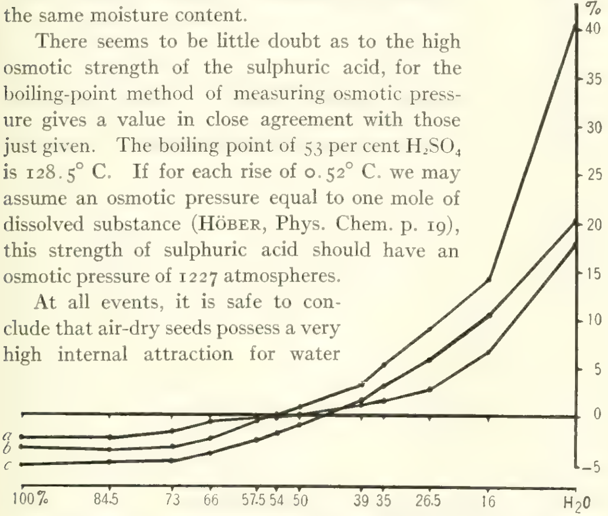

Fig. 3.—Curves of moisture equilibrium of seeds suspended over sulphuric acid: *a*, *Ricinus communis;* *b*, *Triticum sativum;* *c*, *Oryza sativa;* abscissae, strength of sulphuric acid; ordinates, moisture intake by seeds in percentage of air-dry weight.

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[1] Seeds sent to Washington were dried in the vacuum oven by Mr. A. B. Campbell. Upper seeds averaged 5.48 per cent of the absolute dry weight, and lower seeds averaged 5.72 per cent of their absolute dry weight.

B. THE SURFACE FORCES OF SOILS.—1. *The Oswego silt loam.*—A number of tests were made with the subsoil of the Oswego silt loam, the results of 4 of which are shown in table V. The air-dry soil apparently holds its moisture with about the same force as do air-dry seeds, a result one might expect, since both are in moisture equilibrium with the same atmosphere at air-dry.

TABLE V

RELATION OF SOIL MOISTURE IN SUBSOIL OF OSWEGO SILT LOAM TO WATER INTAKE BY SEEDS OF *Xanthium pennsylvanicum*

Soil H_2O in percentage of absolute weight	Intake H_2O in percentage of air-dry weight	Soil H_2O in percentage of absolute weight	Intake H_2O in percentage of air-dry weight
I		III	
6.66 (air-dry)	0.97	5.95 (air-dry)	0.38
7.85	3.35	6.15	1.58
8.92	6.25	7.01	3.73
10.26	9.58	8.68	6.16
11.79	11.94	10.32	10.76
12.74	17.46	11.60	15.79
14.78	20.62	13.16	21.36
16.06	34.00	14.88	28.61
17.35	39.77	16.75	33.86
18.07	41.79	18.07	45.15
19.80	46.54	19.34	49.31
II		IV	
5.83 (air-dry)	0.00	4.65 (air-dry)	−0.53
6.23	1.91	6.46	+1.06
8.27	5.18	7.88	3.68
9.16	6.55	9.30	6.47
10.81	9.81	11.16	10.82
13.56	19.93	12.46	15.81
14.23	23.88	13.91	21.11
15.34	31.54	15.18	32.60
17.10	37.70	17.12	41.98
17.93	43.25	18.87	47.26
19.71	43.79	20.04	50.00

As soil moisture is increased, the moisture intake by the seeds increases at a much more rapid rate, until the approaching saturation of the seed begins to cut down the absorption rate. This happens as the soil moisture approaches the wilting coefficient. Reference to fig. 4 will make the relationship of soil moisture content to seed intake clear.

The general situation is strikingly illustrated by table VI, which combines the results of the 4 experiments of table V. While there are a number of discrepancies, as one might expect, it is evident that the method can be used in measuring approximately the forces residing on the surfaces of soil particles at various soil moisture contents. For instance, when this soil contains about 9.36 per cent of moisture (3.5 per cent above air-dry), it is in moisture equilibrium with seeds at 6.47 per cent above their air-dry weight. The seeds attain about the same equilibrium point with saturated NaCl solution, which has an osmotic pressure of 375 atmospheres,

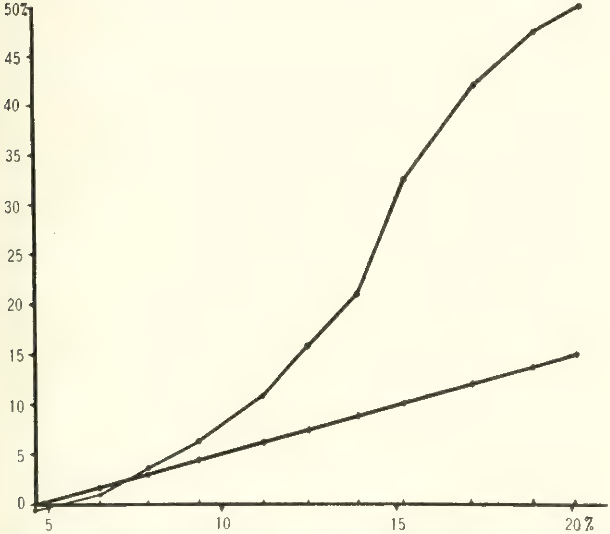

FIG. 4.—Curves showing relation of moisture intake by seeds to increasing soil moisture, plotted from experiment 4, table V; abscissae, percentage of soil moisture in terms of absolute dry weight; ordinates, percentage of increase of soil moisture above air-dry weight for the soil moisture curve (straight), and percentage of intake by seeds in terms of their air-dry weight.

from which it follows that the surface force of the soil particles at the given moisture content is also approximately 375 atmospheres.

TABLE VI

RELATION OF SOIL MOISTURE TO INTAKE BY SEEDS; DATA OF TABLE V COMBINED

Soil moisture in percentage of absolute weight	Intake by seeds in percentage of air-dry weight	Osmotic pressure equal to surface force in atmospheres
4.65 (air-dry)	−0.53	
5.83 (air-dry)	0.00	LiCl saturated = 965 atmospheres
5.95 (air-dry)	+0.38	
6.66 (air-dry)	0.97	
6.15	1.58	
6.23	1.91	(697)*
6.46	1.06	
7.61	3.73	(532)
7.85	3.35	
7.88	3.68	
8.27	5.18	
8.68	6.16	(418)
8.92	6.25	
9.10	6.55	
9.36	6.47	NaCl saturated = 375
10.26	9.58	
10.32	10.76	
10.81	9.81	
11.16	10.82	
11.60	15.79	
11.79	11.94	4 M.NaCl = 130
12.46	15.81	
12.74	17.46	
13.16	21.36	2 M.NaCl = 72
13.91	21.11	
14.23	23.88	
14.78	20.62	
14.88	28.61	M.NaCl = 38
15.18	32.60	
15.34	31.54	
16.06	34.00	M.$C_6H_{12}O_6$ = 22.4
16.75	33.86	
17.10	37.70	0.5 M.NaCl = 19
17.12	41.98	0.4 M.NaCl = 15.2
17.35	39.77	
17.93	43.25	0.3 M.NaCl = 11.4
18.07	41.79	
18.07	45.15	0.2 M.NaCl = 7.6
18.87	47.26	0.1 M.NaCl = 3.8
19.34	49.31	
19.71	43.79	
19.8	46.54	
20.04	50.00	
	51.44	Saturated = 0.00

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* Values in parenthesis calculated from the curve of moisture-holding power of the soil as determined by the known values.

When the soil moisture reaches 6 per cent above air-dry, the moisture intake by seeds indicates a force equivalent to 4M. NaCl solution, which is estimated to exceed 130 atmospheres. At 11 per cent above air-dry the holding power of the soil has fallen to 22.5 atmospheres approximately.

In this manner, comparing the percentage intake from the soil with that from the solutions, as given in table III, one may estimate the surface force for any given moisture content of the soil, each soil type, of course, having specific relations. If it were possible to make absolutely accurate determinations for several points in the curve of moisture intake by seeds, as related to the curve of moisture increase in a particular soil, it would be a simple mathematical problem to calculate the exact water-holding power of the soil particles at any soil moisture content whatsoever for that soil.

2. *The no. 2/0 sand.*—By preliminary tests ranging from 17.5 per cent to 1 per cent of moisture it was found that there was no significant water-holding power in this sand until the moisture content fell to less than 2 per cent. At 1 per cent of soil moisture the seeds took in over 45 per cent of their own dry weight.

The results of a series of tests running from air-dry (0.14 per cent) to a little beyond the wilting coefficient (1.3 per cent) are shown in table VII.

TABLE VII

RELATION OF MOISTURE IN NO. 2/0 QUARTZ SAND TO MOISTURE INTAKE OF *Xanthium* SEEDS

Soil H_2O in percentage of absolute weight	Intake H_2O in percentage of air-dry weight
0.14 (air-dry)	−0.306
0.159	+1.407
0.175	5.02
0.203	21.81
0.44	33.98
0.81	42.40
1.03	45.64
1.49	47.46
1.79	52.06
2.14	72.85*

*Four seeds showing incipient germination, hypocotyls averaging 3 mm. long.

In this sand the very rapid decrease in the force with which its particles hold water as moisture content increases stands in sharp contrast to the slower decrease in the heavy clay subsoil. While the air-dry sand gives the same kind of result as the air-dry clay, by the time the sand contains 0.44 per cent of water the seeds secure as much water as they do in a molecular solution of non-ionizable salts. This indicates a force of 22.4 atmospheres.

3. *Various soil types.*—The results obtained with the subsoil of the Oswego silt loam and the no. 2/0 sand suggested that there might be a general relationship between soils and seeds as regards the amount of moisture seeds will absorb at the wilting coefficient of the soil, whatever value the wilting coefficient might have. To clear up this point, the soil types of table II were used. Each soil was brought as nearly to the wilting coefficient as possible by addition of water. The closeness of the experimental conditions to the wilting-coefficient determinations is shown in columns 3 and 4 of table VIII.

TABLE VIII

RELATION OF WILTING COEFFICIENT TO MOISTURE INTAKE BY SEEDS

Soil types	Percentage of hygroscopic moisture	Percentage of wilting coefficient	Percentage of soil H_2O	Percentage of seed intake
1. Sand (coarse)........	0.205	0.73±0.02	0.65	34.44
2. Loam................	3.130	12.93±0.05	12.66	49.02
3. Sandy loam (very fine)	1.836	8.33±0.08	7.86	48.38
4. Loam................	2.280	12.41±0.02	13.30	49.01
5. Clay loam...........	3.820	16.12±0.01	16.01	49.49
6. Clay loam...........	5.210	16.34±0.02	17.78	47.31
7. Fine sand...........	0.750	3.21±0.03	3.19	49.77
8. Sand (coarse)........	0.218	0.83±0.01	0.80	40.98
9. Loam................	2.30	10.82±0.06	10.51	50.42

The percentage of moisture taken up by dry seeds placed in each soil is shown in the last column of the table. With the exception of the two sands from Yuma, Arizona, which are coarse, the results are fairly uniform. In two cases the soils adhered badly to the seeds, making accurate work very difficult; but corrections were made as carefully as possible. In all the other soils the seeds remained clean, or were easily brushed free of adhering particles. The average intake for the 7 types, excluding the coarse sands, is approximately 49 per cent, and this average agrees rather closely with the intake in the Oswego silt loam subsoil and in the no. 2/0 sand at their wilting coefficients. This probably means that the wilting coefficient represents a fairly definite water-holding power for the soil particle, regardless of its size. By comparison of the results given in table VIII with those given in tables III and VI, it is seen that the "back pull" of the soil particles, the force with which they withhold water from seeds and plants at this critical moisture content, is not more than the equivalent of 0.1M. NaCl solution, or 3-4 atmospheres. This value is surprisingly low.

V. Discussion

In this paper the term internal forces of seeds is used to designate the resultant of all forces within the seed tending to cause intake of moisture; and by surface forces of soils is meant the resultant of all the forces of adhesion, cohesion, surface force, etc., as determined by size, chemical composition, and character of the surface of the soil particles, which cause the soil to retain moisture.

In order to make clear the nature of the problems involved in this work we shall first consider rather fully the moisture relations between seeds and their environment, and then the moisture relations of soils to seeds and to the root hairs of living plants. A careful study of the moisture relations existing between organic bodies, like seeds, and the atmosphere, the soil, and osmotic solutions, if the seeds have semipermeable coats, convinces one that the entrance or exit of water from the seed is due to the interplay of such internal forces as capillarity, imbibition force, colloid hydration forces, etc., with external forces such as atmospheric evaporation, the surface forces active on soil particles, osmotic pressure, etc., according to the environment of the seed. Moisture flows into or out of the seed as one or the other of these sets of forces is the greater. Movement of water continues only until the two forces, unequal at the start, become equal. This establishes moisture equilibrium, and further movement of water must be consequent to some disturbance, external or internal, of the balanced condition of forces. Moisture equilibrium may obtain at any moisture content of the seed, if only the two forces are equal.

The attempt to measure the internal forces of seeds with semipermeable coats by means of osmotic solutions was based upon this conception of the moisture relations, and on the assumption that the total osmotic pressure of the solution is transmitted through the membrane as force when pitted against the internal forces of the seed through the agency of the semipermeable coat. The results of this attempt have been highly satisfactory, and I can see no good reason for doubting that the internal forces of air-dry seeds are approximately equal to 900–1000 atmospheres.

The vapor pressure method, using sulphuric acid, involves another assumption whose validity may be a little more questionable. It is generally admitted that the osmotic pressure of a solution can be calculated with some degree of accuracy from its vapor pressure. I have assumed in addition that the vapor pressure of the seed hydrogels measured against the vapor pressure of strong solutions can be used as a measure of the internal forces of the seed. The assumption seems reasonable enough, but it would be difficult to offer definite proof of its validity at present.

The principal difficulty with the vapor pressure method is the uncertainty as to the osmotic pressure of sulphuric acid. It may seem quite unwarranted to some even to think of estimating the osmotic pressure of strong sulphuric acid by means of a formula, knowing as little as we do of all the factors which enter into the problem in this case, and knowing that these formulae have all been developed for the dilute "ideal" solutions. I realize fully the danger of basing any conclusions upon results obtained by such precarious methods, and offer the results merely for their suggestive value. The formula I have used for calculating the osmotic pressure from the vapor pressure is that given by WALKER (**35**), with S representing the density of the solution rather than that of the solvent. This is necessary in order to make the formula fit a concentrated solution with high density. This same formula has been used by others for the same purpose, as by DRABBLE and DRABBLE (**12**).

Attention is called to a difference in the formulae given by NERNST (**24**) and WALKER. In WALKER's discussion, $\frac{f-f^1}{f}$ represents *relative lowering of the vapor pressure* of the solution as compared with that of water. His formula actually gives the relative lowering. NERNST, however, uses $\frac{p-p^{1}}{p^{1}}$ for this factor in the equation, and with concentrated solutions it is quite a different thing. In a concentrated solution, where the relative lowering of the vapor pressure is 0.75, NERNST'S formula will give a value for the osmotic pressure 4 times as great as that given by WALKER'S formula.

In the third English edition of NERNST he gives an equation for this calculation which he claims gives a very exact value for the osmotic pressure from vapor pressure. His equation is as follows:

$$P = \frac{0.0821 \,.\, T \,.\, 1000\, S}{M} \,.\, ln \frac{p}{p^{1}}$$

The physical chemists claim that this formula should hold in so far as it includes the factors involved. But even this exact formula does not take care of the change in volume occurring on dilution of the acid, nor for the heat of dilution, which is very considerable in the strong acid solutions over which equilibrium of moisture vapor was obtained in these experiments.

I have chosen to use WALKER'S formula because it actually gives the relative lowering of the vapor pressure, as it is supposed to do. However, it is perfectly clear from this discussion that the osmotic pressure of sulphuric acid of any given concentration, especially of high concentration, cannot be measured accurately by any single vapor pressure formula suggested to date.

The measurements made over sulphuric acid give values, therefore, which are merely suggestive. They indicate that the internal forces of air-dry seeds of all kinds are very high, and confirm the high values obtained by the osmotic solution method. But the latter method only, I believe, can be relied upon for the measuring of the internal forces of seeds until the vapor pressure relations are more perfectly understood.

The main conclusion in regard to these seed measurements may be stated briefly thus: It is possible, with seeds having a perfectly semipermeable coat, to measure the water-attracting internal forces residing in the seed substance at any moisture content between saturation and air-dry. The actual values for this range in *Xanthium* seeds are given in the last column of table III.

The results obtained by measuring the soils with measured seeds are of the greatest interest. While it is important to have means of determining in terms of atmospheric pressure the water-retaining power of soils at any degree of dryness between saturation and air-dry, it is still more important to understand the moisture relations of the plant to the soil at and just below the wilting coefficient. Does wilting occur because capillary soil forces and osmotic root forces reach equilibrium? If so, why should BRIGGS and SHANTZ (**8**) have found a uniform wilting coefficient for all kinds of plants when we know that root cells vary somewhat in osmotic concentration from species to species? And why should this uniformity fail in the presence of intense evaporation, as shown by CALDWELL (**9**), and by SHIVE and LIVINGSTON (**32**)? These questions, and the discrepancies between the excellent work done at Washington and at Tucson can probably be answered intelligently, and explained in the light of these experiments.

The moisture equivalent of a soil is the percentage of water left in the soil after centrifuging for a certain time under a force of 1000 gravities, a force about equal to one atmosphere. We have been accustomed to find the wilting coefficient empirically, or to divide the moisture equivalent by 1.84. But we have not known how much greater are the soil forces at the wilting coefficient than at the moisture equivalent. From the results obtained, the pressure value of the wilting coefficient seems to be about 4 atmospheres.

As the soil becomes drier and drier, the forces become greater and greater, until on the approach of air-dry conditions a very small change in moisture content makes a very large change in the forces involved. The increase of the force with decreasing soil moisture is shown graphically in fig. 5, which shows the curve of increasing force for the Oswego silt loam subsoil, and for no. 2/0 sand. The difference in the curves for clay and sand is striking. In the clay, where the surface films are relatively thick, the force decreases very slowly for a considerable distance. But as the films become very thin, the surface force and forces of adhesion of water to particles become very great. This force would reach its maximum presumably when the particles were surrounded by a film of water just one molecule thick. The curve for the sand is very similar, except that the period of slow increase of the soil forces is very short, and that the whole curve lies much nearer to the absolutely dry condition. These relations, of course, are conditioned mainly by the size of the particles.

Let us see now how the soil forces at the wilting coefficient compare in value with the average osmotic pressure of the root hairs. A few years ago Hill (18) showed that the root hairs of *Salicornia* can in a measure accommodate themselves to changes in the osmotic concentration of the surroundings, through increase or decrease of the cell sap concentration parallel to the changes in the environment. That the cell sap of leaves and other exposed parts increases in concentration with xerophytic habitat has been shown by Drabble and Lake (13), Fitting (14), and others. The general conclusion reached by Drabble and Drabble (12), that the osmotic strength of cell sap varies with the physiological scarcity of water in any area, seems most reasonable, and it doubtless holds true of root cells under xerophytic soil conditions. As the soil becomes deficient in water supply, and the surface forces are rapidly increasing in magnitude, we might then expect this increased force of the soil to be paralleled by increase in the osmotic forces of the root hairs, owing to progressive concentration of the cell sap. Such increase might amount to many atmospheres.

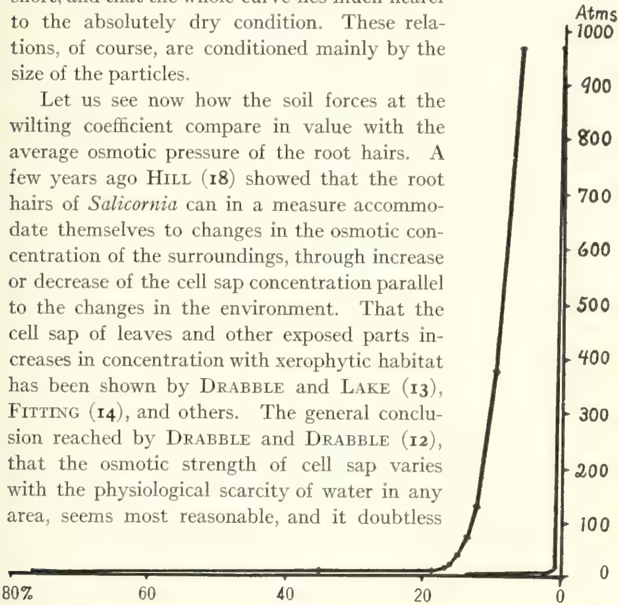

Fig. 5.—Curves showing increase in the surface forces of soils as drying proceeds; to the left, for subsoil of the Oswego silt loam; to the right, for no. 2/o sand.

The most careful, extensive, and valuable study of the osmotic pressure of the sap of root cells under ordinary conditions of soil moisture which has been made up to the present is that by HANNIG (15). He finds that the average root cell sap pressure for 64 species of plants is 0.21M.KNO_3, or equivalent to 7 or 8 atmospheres. It is seen, therefore, that the water-holding power of the soil at the wilting coefficient is only about half that of the average osmotic pressure of the sap of the root cells. Certainly wilting at the wilting coefficient cannot be due to lack of water, for seeds come within a few per cent of taking up as much moisture at the wilting coefficient as when placed in water itself. Nor can it be due to equalization of forces between root hair and soil water, for there is still a gradient of 4 atmospheres of force in favor of the plant. Moisture and gradient for movement of water toward the plant are both present, and yet the plant wilts!

Even in cases where the soils are drier than the wilting coefficient, the accommodation of the root hairs mentioned above would probably maintain this gradient of a few atmospheres in favor of moisture intake. This idea is strongly supported by unpublished work of Miss EDITH A. ROBERTS, who, working in this laboratory, has shown that seedlings of mustard and radish grown in sugar solutions develop root hairs with osmotic pressures usually about 4 atmospheres in excess of the medium in which they grow, the same amount of excess as this gradient at the wilting coefficient. This relationship of internal to external forces was maintained, in her work, up to volume molecular solutions of cane sugar. It is exceedingly probable, therefore, that as soils dry out beyond the wilting coefficient the root hairs maintain an osmotic pressure a few atmospheres in excess of the soil forces until those forces become relatively very high. Nevertheless, permanent wilting occurs within a narrow range of soil moisture under moderate conditions of evaporation.

There seems to be but one reasonable explanation for this situation. The wilting of plants at the wilting coefficient of the soil must be due to the failure of water movement from soil particle to soil particle, and from these to the root hairs, rather than from lack of moisture or gradient. This does not mean complete cessation of movement of film water toward the plant. It is a question of rates. Evaporation continues from the leaves in accordance with atmospheric conditions somewhat regardless of conditions below the soil surface. At the wilting coefficient the film water becomes so stable, and the friction of movement becomes so great, that the rate of movement of water toward the root is quite inadequate to meet the needs of the plant, and permanent wilting ensues.

It becomes clear at once why Briggs and Shantz, working under rather uniform conditions of evaporation, found the same wilting coefficient for all kinds of plants in a given soil, regardless of variability of root sap concentration and other variable factors, for these variables do not affect the point at which capillary movement of water over the soil particles ceases to be effective for the plant. This is determined by the physical properties of the soil, the fineness of the particles being the chief factor. We should expect, therefore, this uniform behavior under moderate conditions. On the other hand, when the evaporation rate is very intense, the plant might be caused to wilt permanently before this wilting coefficient is reached, owing to the fact that after all it is a question of rates. The rate of movement in the soil fails to be adequate sooner.

In concluding this discussion, may I suggest that the methods used and the conclusions reached in this work should receive very critical consideration by plant physiologists, soil physicists, and all others interested in these problems. We have lacked even the most elementary facts concerning these important moisture relations of the soil. This is a first attempt to throw light upon an unexplored region of soil physics. It is hoped that other methods may be devised for testing the correctness of the conclusions reached by the methods presented here. The apparatus is quite simple and easily used. If the results obtained can be fairly substantiated by other methods, the method will be exceedingly valuable in physiological and ecological investigations of many kinds.

VI. Summary

1. The force with which the seeds of *Xanthium pennsylvanicum* absorb water has been measured by two methods: (*a*) osmotic

solutions, and (*b*) vapor pressure equilibrium. The osmotic method is at present the more reliable.

2. The air-dry seeds of *Xanthium* show an initial attraction for water of nearly 1000 atmospheres.

3. The attraction which exists at any moisture content of the seed between air-dry and saturation can be approximated. Table III gives the data.

4. The seeds have in turn been used to measure the complex moisture-holding forces of soils, with the following results:

a) The air-dry subsoil of the Oswego silt loam holds its hygroscopic moisture with about the same force as an air-dry seed, that is, about 1000 atmospheres.

b) As the moisture content of the soil increases, the surface force decreases rapidly. When about 3.5 per cent of water has been added to the air-dry soil, the force remaining is about 375 atmospheres. When the soil moisture reaches 6 per cent above air-dry in this soil, the moisture is held with a force of 130 or more atmospheres. At 11 per cent above air-dry the holding power has fallen to 22.4 atmospheres.

c) At the wilting coefficient of the soil (13.3 per cent above air-dry in the Oswego silt loam subsoil) the "back pull" of the soil particles amounts to not more than that of a 0.1M. NaCl solution, that is, not more than about 4 atmospheres. This is shown to hold true for a number of types of soil with widely varying wilting coefficients.

5. This water-holding power of soils at the wilting coefficient is less than the osmotic pressure of the root hairs of many kinds of plants, as shown by HANNIG and others.

6. The wilting of plants at the wilting coefficient of the soil cannot be due to lack of moisture in the soil, nor to lack of a gradient of forces tending to move water toward the plant.

7. The view is held, therefore, that the wilting at this critical soil moisture content must be due to the increasing slowness of water movement from soil particle to soil particle, and from these to the root hairs, the rate of movement falling below that necessary to maintain turgidity of the cells of the aerial parts, even under conditions of low transpiration.

My thanks are due to Professor L. E. CALL, of the Kansas State Agricultural College, Manhattan, for sending me the subsoil of the Oswego silt loam used in these experiments, and to Dr. LYMAN J. BRIGGS and his assistants in the biophysical laboratory of the Bureau of Plant Industry for making determinations of the moisture equivalent of the local soils, hygroscopic determinations of the seeds in the vacuum driers, and for the various soil types which were so kindly sent to me. I desire also to express my appreciation of the generous way in which the Hull Botanical Laboratory has provided all needed facilities for the work, and especially my indebtedness and gratitude to Dr. WILLIAM CROCKER for much helpful advice and encouragement received during the progress of the work.

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THE VASCULAR ANATOMY OF PIPER METHYSTICUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 218

(WITH TWENTY-THREE FIGURES)

RACHEL E. HOFFSTADT

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(WITH TWENTY-THREE FIGURES)

Historical

LINNAEUS placed the Piperaceae in the monocotyledons among the Aroidaceae; JUSSIEU held that they were near the Urticaceae; KUNTH (8) and BLUME ranked them with the monocotyledons. DE CANDOLLE (4), however, following JUSSIEU, stated that the embryo characters indicated their dicotyledonous character. ENGLER and PRANTL (5) put the tribe Piperales among the lower Archichlamydeae. Included in it are 4 families: Saururaceae, Piperaceae, Chloranthaceae, and Lacistemaceae. The genus *Piper* contains over 1,000 species. MIQUEL (10) separates 7 species from the original classification on the basis of the presence or absence of his so-called "stipula oppositifolia" and persistence of the petiole. Among these is *Piper methysticum* Forst., which he calls *Macropiper methysticum*. ENGLER and PRANTL (5), using as a basis of their classification of these two genera the method of branching in the floral regions, retain the name of FORSTER.

Piperaceae have attracted much attention because of their anomalies, both in anatomical and reproductive features. The former have been described in many species. MIQUEL (10) asserted that the internode of the stem ends blindly in the petiole, and the next internode develops from a bud between the petiole and the end of the foregoing node. He stated also that there are alternate, opposite, and whorled leaves on the same branch. The latter he concluded were atrophies, and the number of internodes which would have been produced would have been the same number as the whorls of leaves. SANIO (11) described in detail the structure and the course of the bundles in the stem of *Peperomia blanda*. SCHMITZ (12) verified the work of SANIO in *Peperomia*. He concluded that the peripheral bundles in *Piper* were joined at their base with the inner bundles of the stem. The small bundles of the leaf trace come from branches of the peripheral bundles. They, as well as the central bundles, go with or without branching into the petiole, which is merely a continuation of the stem.

In an elaborate discussion of the vascular anatomy of the Piperaceae, WEISS (**19**) deals with 11 species of *Peperomia* and 8 of *Piper*, and draws the conclusion that in the Piperaceae there is a transition from the monocotyledonous to the dicotyledonous type of bundle.

By far the most detailed account is that of DE BRAY (**3**). He used 4 species of Saururaceae, 16 species of *Piper*, 3 species of *Artanthe*, and 11 species of *Peperomia*. In *Piper* he found 2-4 rings of bundles arranged in two systems, the peripheral and the pith systems. The peripheral bundles are of two sizes; the outer ring is completed by an interfascicular cambium; the leaf trace comes from a peripheral ring, which in the node below is a branch of the central bundles; the vascular supply of the axillary buds is from both peripheral and central bundles. He compares the families of the group, but shows no relationships.

In VAN TIEGHEM'S (**17**) work on the mucilage canals of *Piper* he describes in detail the stem of *Piper nigrum*. He found that there are two circles of bundles; that the pericycle becomes lignified late in the growth of the stem; that the cambium for a long time cuts off segments only on the side toward the pith, and may cut off very late a few segments on the other side; that the mucilage canals vary in location with different forms and are not continuous through the nodes; and that the canal of the petiole is not continuous with that of the branch.

The present study of *Piper methysticum* was taken up with the idea of giving a modern interpretation to the vascular structure, and to ascertain whether the stem keeps pace with the gametophyte of the group.

Material

I am indebted to Dr. W. J. G. LAND for the material for this study. It was collected on the Island of Tutuila, Samoa (fig. 1). The plant is a native of the South Sea Islands, and is commonly called *Ava* or *Kava*, the natives extracting from its roots the national beverage bearing that name.

It becomes a shrub 1–1.5 m. in height and is propagated mostly by cuttings. No seedlings were obtainable. The leaves are ovate, 18–25 cm. in diameter, with a deep cordate base and a short accuminate apex. The blade is bright green, smooth above, and bears fine multicellular hairs on the veins beneath. It is distinctly petioled, the petiole having a sheathing base and a deciduous stipule. The leaves are alternate and the dense flower clusters are borne opposite them. The internodes are 7–10 cm. long, the nodes being swollen, smooth, and in the older ones irregularly spotted. Both old and young stems have an herbaceous appearance. The largest nodes examined were 2.75 cm. in diameter, and the smallest were 2 mm.

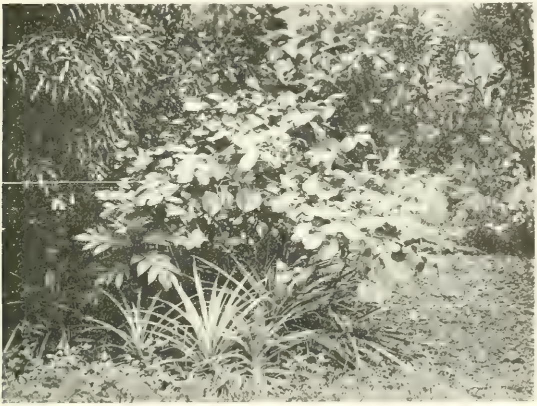


FIG. 1.—*Piper methysticum* growing in Samoa

For the sake of comparison *Piper umbellatum* (*Heckeria umbellata* Kunth) was used, the material being obtained from plants brought from Mexico. In shape and size the leaves resemble those of *Piper methysticum*. The petiole has a sheathing base;

the nodes are swollen and marked with several distinct rows of spots; the internodes are 5–9 cm. long and when young are covered with dense rows of hairs, giving a ridged appearance; the older internodes are smooth but spotted.

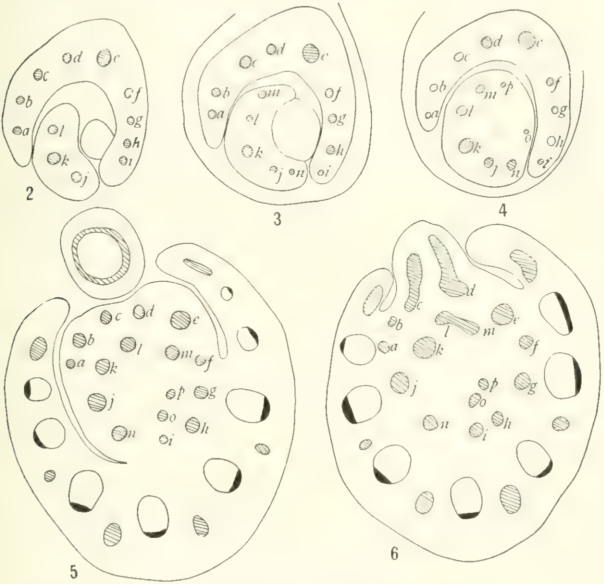
The younger stems and nodes were cut in paraffin at 25–30 μ and complete series obtained. The largest stems and nodes were treated with hydrofluoric acid for one week and then sectioned at 30–50 μ with a sliding microtome, in this case also the series being complete. The safranin Lichtgrün stain was used.

Origin and course of stem bundles

The stem of *Piper methysticum* contains at maturity three rings of bundles, one peripheral and two within the pith, the bundles being foliar. The terminal bud is enveloped in the sheathing base of the youngest leaf. In some cases a bud was found within the sheath of a single leaf with as many as 3 leaves, each of which was inclosed in the base of the petiole just beneath it.

The leaf primordium appears on the stem tip early and forms about it a semicircle, and 5–7 procambial strands are soon differentiated. These connect with the first strands of the stem (figs. 2–4, *j–p*). Strands *j–p* are the strands of the youngest leaf (node 1); *k* is the first to be differentiated; *j* and *l* follow quickly; *o* and *p* come last. The bundles of the second leaf (node 2) are represented by the series *a–i*. At node 1 the stem contains a single row of scattered bundles. These bundles (*j–p*) become the inner bundles of the stem at node 2, and *a–i* of leaf 2 become the peripheral bundles (fig. 5). Through the following internode these two sets of bundles run approximately parallel and become the inner rings of the next node (node 3, fig. 6). The union of the two inner rings takes place here. Five methods of joining were observed in the case of the undifferentiated strand: (1) two strands from the same ring may join (fig. 8, *lm*); (2) two strands from different rings (fig. 7, *ak*); (3) one strand may divide and join a strand of a different ring (fig. 7, g^1p); (4) two strands from the same or different rings may unite and then join a branch of a strand from either ring (fig. 9, *efg*, *ino*); (5) three strands may join, the result of this, for a short time, being a single inner ring (figs. 9, 10, *dlm*).

The peripheral bundles at any node may branch. In older nodes this branching may occur just before the peripheral bundles become pith bundles, and part remain in the peripheral ring; the latter will then connect with the bundles of the leaf at the node below (fig. 19).



FIGS. 2–6.—Transverse section through a terminal bud of *P. methysticum*, showing the course of the bundles; ×168.

The coalescence of the branches of these bundles does not always occur at the node, but may occur above or below it, as follows: (1) branches of the same bundles may join before passing through a node; (2) branches from different bundles may unite; (3) branches of a peripheral bundle of the internode above may join a peripheral bundle of the internode below. Not infrequently strands isolated in the anastomosing of the pith bundles at any node may join the peripheral bundles at that node. The peripheral bundles of any internode, therefore, when young will consist of one set of foliar bundles only; and when older may contain portions of the foliar bundles of the leaves of 3 nodes.

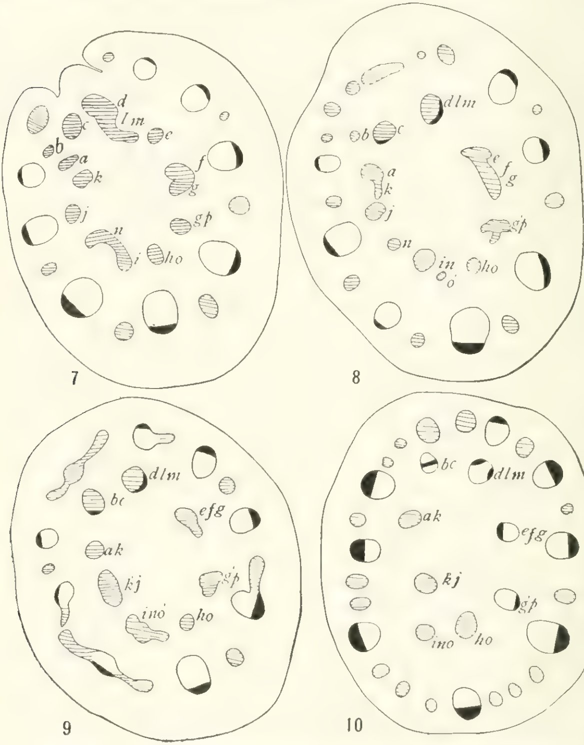


FIG. 7–10.—Transverse sections through a terminal bud of *P. methysticum* showing course of bundles; sections follow those of figs. 2–6; ×168.

The node in which the strands have become differentiated presents a most complicated appearance. One often finds for a short time xylem entirely surrounding phloem, and vice versa. Bundles may join with either the phloem or xylem together. They may lie side by side and union may occur either in the xylem or in the phloem. Not infrequently the xylem of two bundles may unite, and the phloem of a third join the phloem of either of the others. Strands become separated from bundles and several of these may coalesce to make a bundle, or they may join with other bundles. One often gets cross-sections and longitudinal sections of the same bundle in the same section.

The bundles in one internode are in the periphery, and through the following two internodes are pith bundles. The position in the pith often gives the appearance of two complete rings and one-half of a third ring, when the number of bundles is large; but careful study shows that there are really only two rings. The number of bundles reaches 45–50 in the pith.

Description of stem in detail

As previously stated, the stem of *Piper methysticum* has two sets of bundles which may be designated as the peripheral ring and pith or medullary rings, two in number, and an irregular half-ring which gives the appearance of a third ring at times. The bundle type is open collateral endarch, oriented for the most part in the usual manner. Sometimes, however, one finds the protoxylem points appearing in reverse orientation (fig. 10, *e–g*). Union is often incomplete and one finds double bundles persisting in the internode, and these may be of two types. The xylem points or the phloem points may be together (figs. 11, 12).

The peripheral ring of bundles is formed of two sizes: the primary, those differentiated first; and the secondary, termed supplementary by DE BRAY (**3**) (fig. 13). The secondary bundles are branches of the primary bundles and lie between them. More than one primary bundle may contribute to a secondary one (fig. 9); that is, two bundles may branch and the branches join. The number of secondary bundles varies from none to 3, according to the age and size of the stem. Near the tip there are none (fig. 4), as observed by SCHMITZ (**12**).

The inner rings of bundles are approximately the same size. This does not differ from the majority of the species of *Piper*.

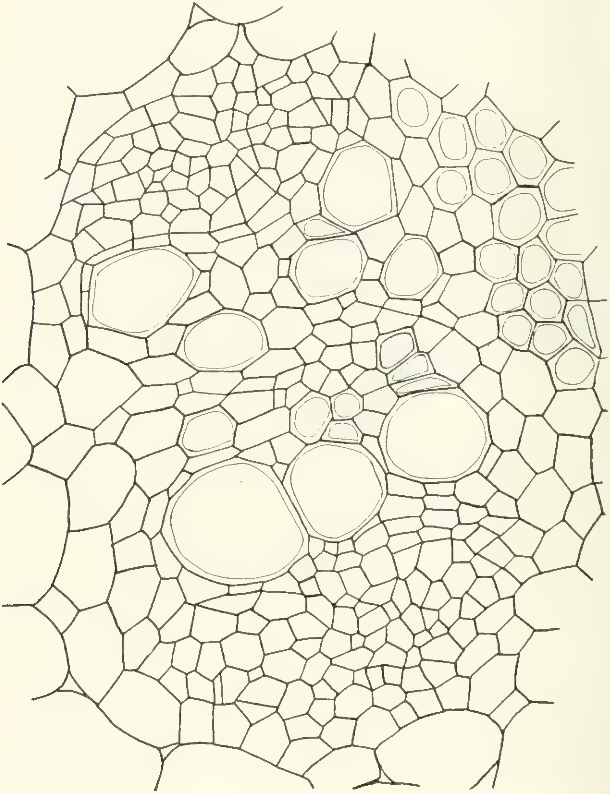


FIG. 11.—Detail of a double bundle with phloem on the outside and lignified tissue at the side; ×820.

Between the pith and the peripheral ring of bundles in the young stem is a continuous band of parenchyma, 4–6 rows thick, which early becomes lignified (figs. 13, 14). Later, owing to growth of the bundles, this band is broken and patches appear only at the inner portions of the bundles (fig. 18).

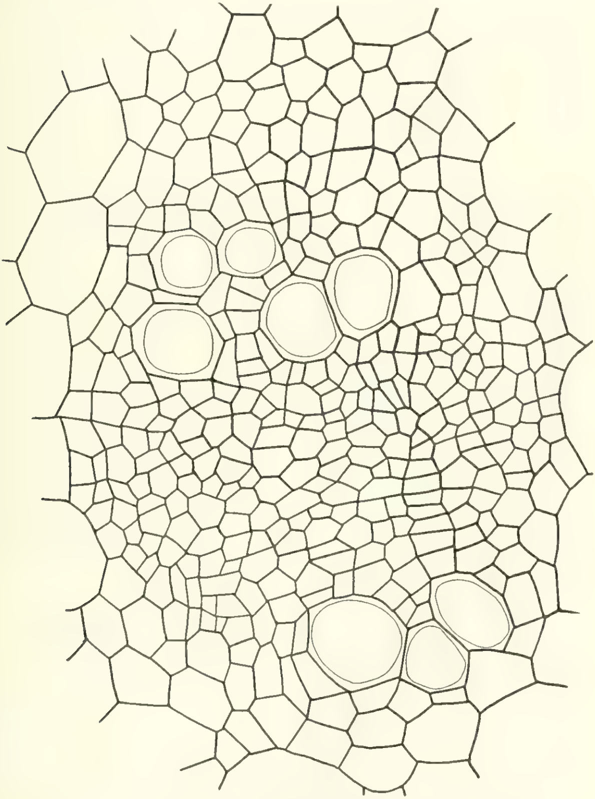
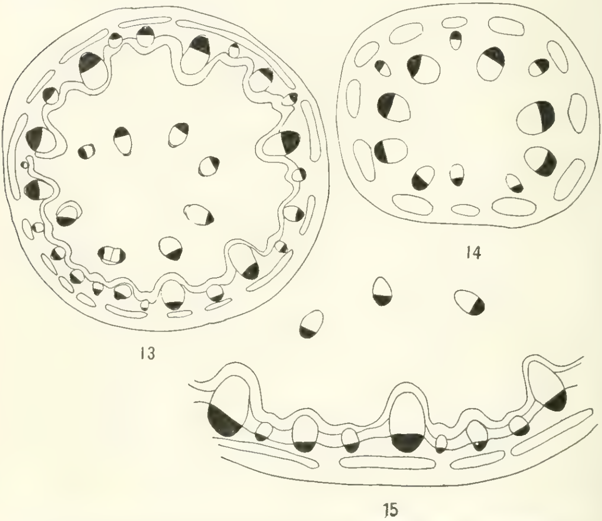


FIG. 12.—Detail of double bundle with xylem points outside; ×820

The pericycle consists of only a few cells (fig. 16) just outside the bundles in both the peripheral and pith systems, and nowhere else. It can be distinguished easily in the young stems by the dense cell contents. Late in the history of the plant the pericycle becomes lignified. There is no differentiated endodermis in either old or young stems.

In the cortex of the young stem is a continuous band of tissue 7–8 cells wide, which responds to the test for suberin (TUNMANN **16**). This is in patches in the older stems. The intercellular spaces of the pith are very large.



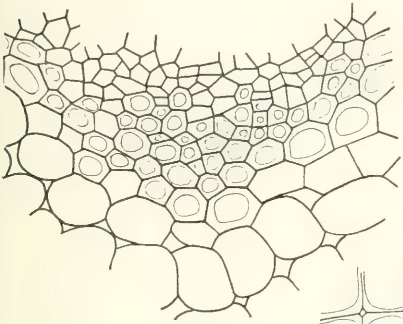
FIGS. 13–15.—Fig. 13, young stem of *P. methysticum* after differentiation has been completed, ×80; fig. 14, petiole, ×101; fig. 15, sector of stem after interfascicular cambium has been developed, ×101.

Secondary activity in the stem

There are two methods by which the stem of *Piper methysticum* enlarges: (1) by active division of pith cells; (2) by cambail activity.

1. In the center of and at irregular intervals throughout the pith, groups of cells will divide actively throughout the internode.

2. Each bundle of both the peripheral and pith rings has a cambium. The inner ones do not enlarge greatly. The outer or peripheral bundles lay down a ring of wood often 4-5 mm. in thickness. The proportion of xylem to phloem is about 8:1 (fig. 18).



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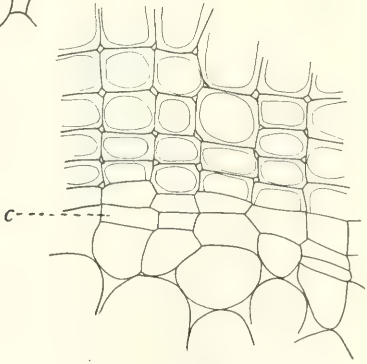

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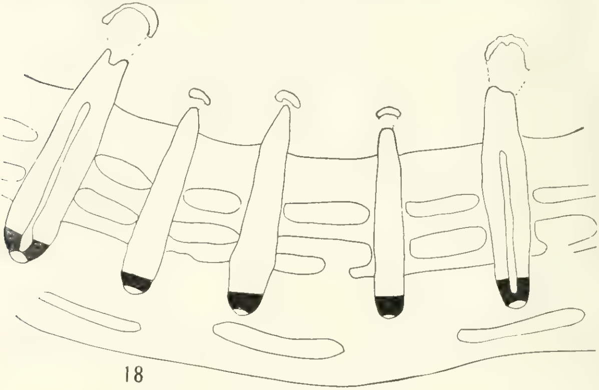
FIGS. 16, 17.—Fig. 16, detail of pericycle of *P. methysticum;* fig. 17, detail of medullary ray; *C*, cambium; ×820.

Late in the history of the stem an interfascicular cambium appears (fig. 15), which cuts off segments on the inside only; very late a few segments may be cut off on the other side (fig. 17). This closes the stem cylinder. The interfascicular cambium appears much later in the nodes and remains active much longer.

VAN TIEGHEM calls attention to the one-sided action of the cambium in his work on *Piper nigrum.*



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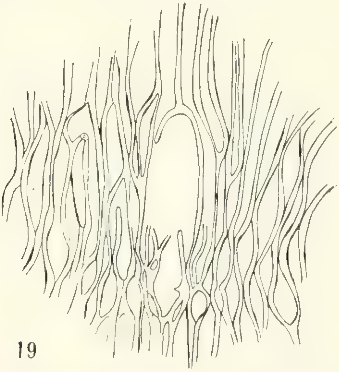

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FIGS. 18, 19.—Fig. 18, section of peripheral ring of the bundles of *P. methysticum* at a mature node, showing banded appearance of the rays, ×35; fig. 19, tangential section of peripheral bundles, showing branch gap; ×7.

The medullary ray becomes lignified and in longitudinal section shows pitted cells. At the node in the largest stems available, cells next to the cambium do not lignify and remain active. These are also scattered at irregular intervals throughout the ray. In section this gives the ray an irregular banded appearance (fig. 18). There are no growth rings. Much of the wood parenchyma touching the pith retains its activity at the node and divides long after secondary wood has been laid down. This pushes the primary vessels of the bundles apart at great distances, as well as the cells of mechanical tissue (fig. 18).

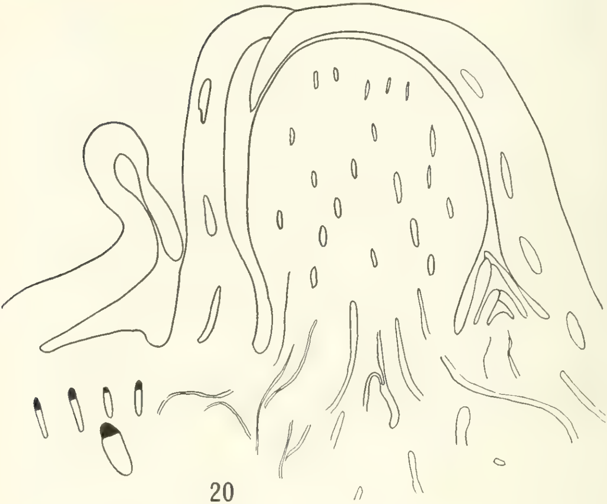
The stem when young is packed with starch and piperin. Mucilage cells are scattered throughout it, and the buds especially are covered with mucilage.

Leaves

The leaves have distinct petioles, the sheathing bases of which extend entirely around the stem, which becomes narrow opposite the point of insertion of the leaf. The leaf traces vary in number from 7 to 14. The bundles may be of two sizes. The lower part of the petiole is flattened on one side; the upper part is circular. The bundles form a crescent in the lower portion of the petiole (fig. 21), this crescent being made complete in the upper portion by the branching of the bundles at the points of the crescent (fig. 14). The secondary bundles form a more or less complete outer ring, are of the same type as those of the stem, and anastomose at any portion of the petiole. Union of the primary and secondary bundles, however, takes place before the traces enter the blade, which has 7–9 strong palmate veins, the 3 median ones running parallel for a while before they swing out laterally. The vernation of the leaf is involute.

Branching

Buds are borne in the bases of the sheathing petiole, the number varying from 1 to 3. The appearance of a single bud is often to one side instead of in the median position, which probably accounts for the appearance of branches coming from the side of the leaf. The buds are invested by a large amount of mucilage. The central bundles of the branch connect with those of the stem, and the peripheral ones connect with the peripheral bundles of the



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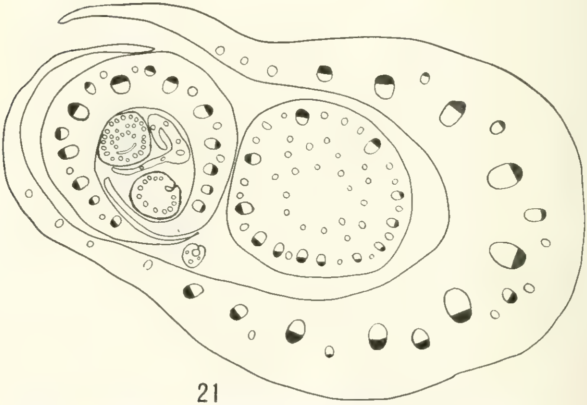

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Figs. 20, 21.—Fig. 20, transverse section of mature and young buds, ×32; fig. 21, transverse section through the base of a petiole, showing branches arising in axils of abortive leaves, ×28.

stem (fig. 20). In two cases it was observed that the branch did not develop in the axil of a leaf where only the blade was developed (fig. 21), which was due probably to the pressure caused by the enveloping sheaths of the preceding leaves.

Piper umbellatum

The stem of *Piper umbellatum* is not essentially different from that of *Piper methysticum.* At maturity it has only one ring of pith bundles. The bundles, instead of traversing two nodes as internal bundles before fusing with the next group, traverse but one (fig. 22). The young plants are covered with hairs, both unicellular and multicellular. The number of leaf traces is 7–9. In the young stem there is an abundance of mucilage scattered in all regions. In the old stem a single mucilage canal appears in the center, which is continuous through the nodes and in the branches; VAN TIEGHEM found none that was continuous through the nodes (fig. 23).

Summary

1. The stem of *Piper methysticum* consists of two systems of bundles, peripheral and pith, the latter being in two rows.

2. The peripheral bundles are of two sizes: primary, the larger; secondary, the smaller. The latter are branches of the former.

3. The bundle type is collateral endarch.

4. The bundles are of foliar origin.

5. The bundles, after entering the stem, remain in the peripheral region through one internode and then traverse the pith for two internodes.

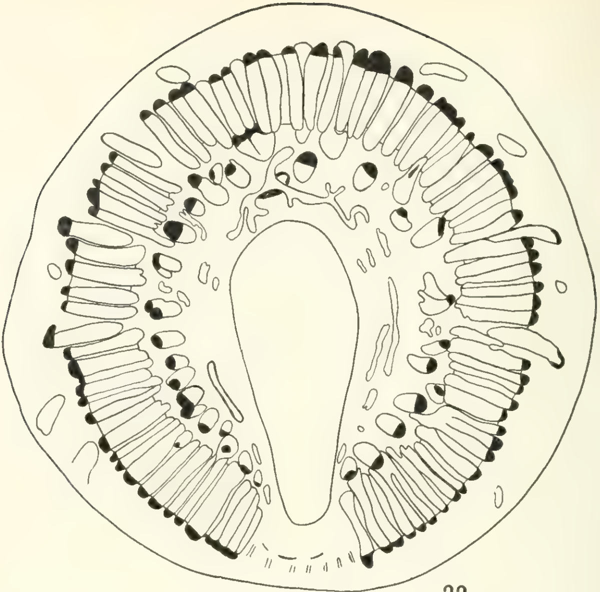
6. Both the peripheral and pith bundles show anastomoses.

7. The pericycle consists of only a few cells outside the bundles, which become lignified.

8. There is no differentiated endodermis.

9. The stem enlarges by cambial activity and divisions in the pith region.

10. An interfascicular cambium appears late in the internode and cuts off segments only on one side; it comes still later in the nodes.



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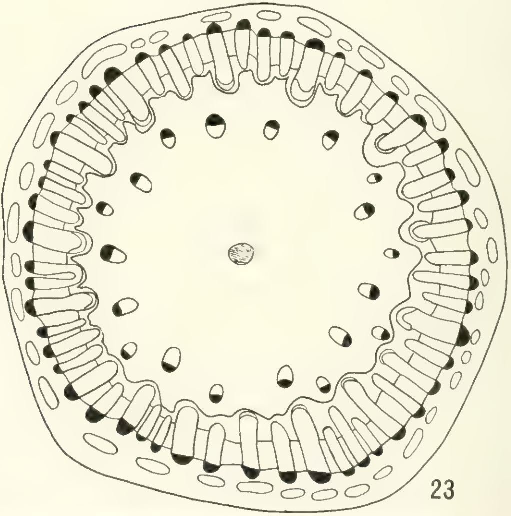

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FIGS. 22, 23.—*P. umbellatum:* fig. 22, section through mature node; fig. 23 section through internode; ×12.

11. Leaf traces are many.

12. The base of the leaf is sheathing and vernation is involute.

13. Buds vary in number and position.

14. *Piper umbellatum* differs from *Piper methysticum* in the following ways: (1) one ring of pith bundles only; (2) the mucilage canal runs through the center of stem and node; (3) the bundles run through one internode only in the periphery and one in the pith before fusing with those of the leaf above.

15. Both stems are packed, especially when young, with starch, piperin, and mucilage.

16. There are no growth rings.

Discussion and conclusions

The anatomy of *Piper methysticum* and of *Piper umbellatum* agree for the most part with the anatomy of the species of *Piper* already described; especially is this true in reference to the work of DE BRAY (**3**; see also DE BARY **2**). VAN TIEGHEM reports a differentiated endodermis in all species of *Piper*, but this was lacking in these forms.

The question of most importance is the relationship shown by these forms. SINNOTT and BAILEY (**13, 14**) assert that the most primitive condition of angiosperms was woody, and that there is a tendency toward the herbaceous habit in the more recent groups. These forms show a tendency toward elimination of secondary growth and a breaking up of the woody cylinder in the following characters: (1) the pericycle is broken and all that remains is a small patch outside of each bundle; (2) the interfascicular cambium appears late in the internode and still later in the nodes; when it does occur the activity is primarily on one side; (3) there is a tendency toward the scattering of the bundles in the pith. These, together with the sheathing base of the petiole, the multilacunar (SINNOTT **13**) type of node, and the anastomosing of the bundles at the side, show a monocotyledonous tendency. Moreover, the embryo sac of the group shows the elimination of one division in oogenesis.

One may conclude from this combination of characteristics that *Piper methysticum* and *Piper umbellatum* lie in a group well toward the level of monocotyledons, and the conclusion is borne out by the female gametophyte.

The writer is indebted to Professor JOHN M. COULTER for suggestions and criticisms, and to Dr. W. J. G. LAND for the material used and for direction and encouragement during the investigation.

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With the regards of the writer.

RESEARCH BULLETIN NO. 8

THE UNIVERSITY OF NEBRASKA

BULLETIN
OF THE
AGRICULTURAL EXPERIMENT STATION
OF
NEBRASKA

A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO

By GEO. K. K. LINK

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A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 219

GEORGE K. K. LINK

(WITH THIRTEEN FIGURES)

There is little doubt among phytopathologists that members of the genus *Fusarium* play an important rôle in producing diseased conditions in many plants, both wild and cultivated. According to WOLLENWEBER (**41**), *Fusarium* spp. produce wilt in members of the following families: Liliaceae, Bromeliaceae, Musaceae, Solanaceae, Convolvulaceae, Leguminosae, Malvaceae, Linaceae, Cucurbitaceae, Cruciferae, Compositae, Araliaceae, Caryophyllaceae, and Pedaliaceae.

History

The genus *Fusarium* was established by LINK (**20, 21**) in 1809, and *Fusarium* species were reported on rotted and ring-discolored tubers by MARTIUS in 1842, HARTIG in 1846, and SCHACHT in 1856. PIZZIGONI (**29**) and WEHMER (**38, 39**) demonstrated by experimental inoculation that *Fusarium* species can bring about tuber rot. They referred to the *Fusarium* in question as *F. solani*. Others, however, among them FRANK (**11, 12**), repeating their work, obtained negative results so far as *Fusarium* species were concerned; while DE BARY (**6**) and many others regarded the *Fusarium* spp. as nothing more than obligate saprophytes.

RESEARCH BULLETIN NO. 9, AGR. EXP. STATION OF NEBR.

The credit of first demonstrating experimentally the relation of *Fusarium* spp. to certain plant wilts belongs to SMITH (34), who found a *Fusarium* responsible for watermelon wilt. SMITH and SWINGLE (35) reported a potato wilt and tuber rot which they considered due to a *Fusarium* which they called *F. oxysporum*. They considered this organism identical with *F. solani* of PIZZIGONI and WEHMER, and used the oldest name available, *F. oxysporum* (SCHLECHTENDAHL, 1824); however, they reported no experimental inoculations. PETHYBRIDGE and BOWERS (28) reported a dry rot due to *F. solani*, and LONGMAN (22) also reported a dry rot due to a *Fusarium*.

Many pathologists and mycologists entertained considerable doubt as to the parasitic nature of *Fusarium* spp., while others were quite convinced of their parasitic nature. SORAUER (36) was quite positive in his decision, while MASSEE (25) wavered. LINDAU (18) remained skeptical and referred to the *F. oxysporum* of SMITH and SWINGLE as a "Mischart." DUGGAR (10) was quite positive in his decision. Much of this difference of opinion undoubtedly was due to the confusion that prevailed as to the status of *F. solani*, *F. oxysporum*, and the genus in general, since no basis for extended morphological study of the genus had been established, and even the genus itself had not been sharply defined. MASSEE (25) considered *F. solani* to be the conidial form of *Nectria solani;* while REINKE and BERTHOLDT (30) considered it the conidial form of *Hypomyces solani*. LOUNSBURY (23) tried to arbitrate the matter by suggesting that *F. solani* and *F. oxysporum* are one and the conidial stage of *Nectria solani*. APPEL and WOLLENWEBER (5) published a monograph in which they defined the genus and brought some order into the chaos of species. Among other radical changes they dropped *F. oxysporum* and established *F. orthoceras* in its place.

MANNS (24) demonstrated by experimental inoculation that a *Fusarium*, which he designated (following SMITH and SWINGLE) as *F. oxysporum*, could produce tuber rot and wilt. He made no morphological studies, however, and undoubtedly had not had access to APPEL and WOLLENWEBER'S monograph.

JAMIESON and WOLLENWEBER (16) published an account of a dry rot of tubers induced by a *Fusarium* which they described as a new species (*F. trichothecioides* Wr.). They refer to it as "a wound parasite capable of destroying potato tubers" and say "this disease is clearly differentiated from the wilt and dry rot ascribed by SMITH and SWINGLE to *F. oxysporum*."

Later, the writer (**19**) submitted his studies of a dry rot occurring among Nebraska potatoes as a thesis to the Graduate Faculty of the University of Nebraska. The work was done at the request of Dr. E. MEAD WILCOX, and consisted in part of a study of the morphology of a *Fusarium* that had been isolated from dry rotted tubers in 1908 by Miss VENUS W. POOL from potatoes that farmers had sent in from throughout the state during the season 1907–1908. Miss POOL established the causal relation of this *Fusarium* to the dry rot by experimental infection, and named the organism in manuscript *F. pulverulentum*, because of its powdery habit of growth. Both field and laboratory work were carried on for several years, and it was found that this organism caused primarily a dry rot of the tuber, and that it was not the *F. oxysporum* of SMITH and SWINGLE, a culture of *F. oxysporum* having been furnished the laboratory for comparative work through the courtesy of Dr. SMITH. The results were to have been published in 1911, and the organism was to be named *F. pulverulentum*, but upon the appearance of APPEL and WOLLENWEBER'S monograph Dr. WILCOX proposed to the writer that he reinvestigate the organism along the lines suggested by these authors. This was especially desirable since *F. oxysporum* had been dropped and several new species established. Not only was this carried out, but the whole etiology was gone over again and all of Miss POOL'S results verified. It was found that APPEL and WOLLENWEBER (**5**) had not described the species, and consequently it was described as *F. tuberivorum* Wilcox and Link (**40**). It was so named because of the apparent restriction of its activity to tubers.

A comparison of this paper and the paper of JAMIESON and WOLLENWEBER (**16**) made it seem quite likely that both were dealing with the same organism. The organism was isolated in the Washington laboratories from potatoes sent in from Washington, Nebraska, and other states in 1910, and WOLLENWEBER upon his arrival in the laboratory, using his monograph as the basis, described it as a new species. He told the writer in 1913 that he felt convinced that we had described one and the same thing. Comparative studies made by the writer during the past year verify this point of view, and since the Nebraska publication by WILCOX, LINK, and POOL (**40**) did not appear in print until 1913, the name *F. trichothecioides* should be adopted.

WOLLENWEBER (**41, 42**) published a further paper in which he categorized the *Fusarium* spp. very sharply, dividing the genus into sections on the basis of physiological (that is, pathogenicity) and morphological (that is, conidia and chlamydospores) characters. *F. oxysporum* was again established and taken as the representative of the section ELEGANS, which comprises vascular parasites; and *F. trichothecioides* was put into the section DISCOLOR, which comprises parenchyma destroyers. He distinguished sharply between these and also between the vascular ring-discoloring *Fusarium* species of section ELEGANS and the tuber-rotting *Fusarium* species of sections DISCOLOR, GIBBOSUM, MARTIELLA, etc.

Referring to the papers by SMITH and SWINGLE (**35**), MANNS (**24**), and others, particularly to that by MANNS, he writes: "They do not separate fusarioses causing tuber rot from those causing both the wilt diseases of the plant and ring discoloration of the tuber, so that the reader might conclude that both wilt disease and tuber rot are caused by the same organism." Referring to his own experiments, he writes: "It also brings out the striking fact that the fungus, a typical xylem inhabitant, does not entirely destroy the tuber without the help of tuber rot *Fusarium* or bacteria," and "the fact that *F. oxysporum* causes the wilt of growing potato plants and only uses the xylem of the stem end of tubers for over-wintering, without producing a rot of the parenchyma, leads to interesting comparisons with the following 4 species which are able to destroy the tuber entirely from artificial wounds, namely, *F. coeruleum* (Lib.), *F. trichothecioides* Wr.," etc., and finally "the fact that the latter (*F. oxysporum*) cannot produce a tuber rot gives a biological contrast to the wound parasites of the tuber, and the fact that they cause the wilt disease of the growing plant presents a contrast to the saprophytes."

Problem and method of attack

In the spring and summer of 1914 the writer discussed the *Fusarium* situation as outlined by WOLLENWEBER with Dr. E. M. WILCOX and Dr. WILLIAM CROCKER. The former suggested that the whole situation ought to be gone over, and the latter that it would be of interest to search for the physiological basis of this alleged biological contrast. It is clear that, if the strict categories of WOLLENWEBER exist, then potato parenchyma must possess either an absolute or an effective immunity toward *Fusarium* spp. of the ELEGANS section, and that *Fusarium* spp. of the DISCOLOR section are either absolutely or practically unable to produce vascular mycoses or wilts.

The purpose of this research was twofold: (1) to determine whether such a sharp biological contrast exists; and (2) to determine what is the physiological basis for such a contrast. Experimental infections of potato plants and tubers were used for the first phase of the problem. It was clear that the second phase might involve a great many considerations, such as the structural, compositional, and metabolic nature of both host and parasite, as well as the relation of environmental factors to these. The important rôle played by the structural and compositional peculiarities of the potato and the influence of external factors upon these is well illustrated by the studies of APPEL and KREITZ (**1, 3**) on the efficacy of the cork layer in checking bacterial invasions of the tuber. Considerations of time and equipment limitations made it obligatory that the scope of the work be limited to a study of a few representative strains of the groups.

The writer is under obligation to the Departments of Agricultural Chemistry, Horticulture, and Experimental Agronomy of the University of Nebraska Experiment Station for the use of materials and equipment; to Miss ETHEL BEATY for help in much of the laborious routine; to Dr. FLORENCE A. MCCORMICK for valuable help in the anatomical and microtechnical phases of the problem; and to Mr. R. A. DAWSON for help in preparing the photographs.

The writer decided to work with *F. oxysporum* as representative of the vascular parasite section (ELEGANS), and with

F. trichothecioides or *F. tuberivorum* as representative of the parenchyma-invading section (DISCOLOR). Since it was desirable that the identity of the organisms be well established, the writer asked Mr. W. A. ORTON, in whose laboratories Dr. WOLLENWEBER had carried out his recent work, for cultures of the organisms. It was impossible to get cultures which had been authenticated by Dr. WOLLENWEBER, since he had gone to war, but through the courtesy of Mr. ORTON, Mr. CARPENTER (Dr. WOLLENWEBER'S assistant) furnished a strain of *F. trichothecioides* (no. 41, 1916) and a strain of *F. oxysporum* (no. 3345A). The other strains of *F. trichothecioides* used had been isolated by the writer in 1911, and were described as *F. tuberivorum*. Several strains of *F. oxysporum* isolated from Nebraska potatoes were also used.

Pure cultures of these organisms were maintained on sterilized rice in plugged Erlenmeyer flasks, and these were used as a point of departure for all the work recorded.

I. Infection experiments

(1) EXPERIMENTAL INFECTION OF TUBERS

Tubers of the Early Ohio and Red Cobbler varieties were used in these experiments. Only sound tubers were selected, and these were thoroughly cleansed and sterilized before infection. At first they were sterilized by the formaldehyde gas method recommended by WOLLENWEBER (**41**). Several difficulties were encountered in using this method. It was found very difficult to remove the last traces of the gas without contaminating the chamber, and the tubers often showed the characteristic formaldehyde vapor injuries that have been discussed in bulletins of the New York Experiment Station (**13**, **37**). Consequently, the writer abandoned the first method and sterilized tubers by immersing in 1:1000 $HgCl_2$ solution for 1.5 hours. Inoculation was carried out by removing a piece of the cortex with a sterile cork borer, placing an infected grain of rice into the hole, and then replacing the piece of tuber tissue. The wound was then sealed with sterile grafting wax and the tuber placed into sterile chambers. This proved an efficient and convenient way of carrying out the great number of experimental inoculations made.

The first inoculations were made in December 1914. The cut ends of 20 Early Ohio tubers were wetted with spore suspensions of *F. trichothecioides* and 5 tubers were kept as controls. Four inoculated and one control potato were kept in each compartment at a temperature of 25° C. in an almost saturated atmosphere. After 4 weeks all of the inoculated tubers were in advanced stages of rot.

On January 31, 3 potatoes were inoculated according to the second method with *F. oxysporum*, and 3 with *F. trichothecioides*, and kept at 20° C. until February 17. Two of the former set were slightly rotted and one totally, while the entire latter set was rotted severely. The controls showed no rot (fig. 1).

On January 15 another series was started which was kept at a temperature ranging from 15–20° C. until February 15. Six sets of 3 tubers each were started and each set was kept in a separate sterile chamber, 2 tubers of each set being inoculated by smearing cut surfaces with agar grown inoculum. Sets I, II, and III were inoculated with *F. oxysporum*, and sets IV, V, and VI with *F. trichothecioides*. In set I, one inoculated tuber was rotted, while the other and the control were sound; in set II, one was deeply rotted and the others sound; in set III, one was deeply rotted and the others sound; in set IV, two were rotted and the control sound; in set V, two tubers were rotted slightly and the control sound; and in set VI, two tubers were rotted and the control sound.

F. oxysporum and *F. trichothecioides* were re-isolated from these rotted tubers by placing tissue cut from such tubers on plated glucose agar. Nothing other than the organism with which the tuber had been inoculated developed. Inoculum from these plates was used in infecting tubers again with the same results.

Since these results were at variance with the statements of WOLLENWEBER the experiments were repeated with hundreds of tubers, and the results were verified.

DISCUSSION.—Tubers inoculated with *F. oxysporum* did not develop the ring discoloration that is considered characteristic of the activity of *F. oxysporum*, but a general rot of the whole tuber. Generally, however, this was not a dry rot, but a rot that resembles more the soft rots of bacterial origin, although it is not accompanied by the offensive odors of bacterial rots, producing a blackening and softening of tissue which extends a considerable distance beyond the actual site of the organism. This was demonstrated microscopically and culturally. At times, however, especially in cold, dry conditions, a dry rot as typical as that produced by *F. trichothecioides* was produced. *F. trichothecioides* invariably produced a dry rot with only a very limited darkened zone extending beyond the destroyed zone, made up of large cavities and a mixture of disintegrated, dry, shrivelled tissue and fungus tissue. No darkening extended beyond the actual site of the fungus and no softening of tissue occurred. Microscopic examination revealed the fact that *F. trichothecioides* attacked the tissue intracellularly and destroyed each cell completely before it proceeded to the neighboring cell, while *F. oxysporum* attacked the tissue intercellularly at first, and then attacked the cells intracellularly, but not until the tissue had been blackened and disorganized. In this way a softened tissue without cavities was produced. These rots produced experimentally with pure cultures of *F. oxysporum* lend support to the observations and conclusions of SMITH and SWINGLE (35), MANNS (24), who reports the occasional appearance of black specks in the parenchyma of tubers infected with *F. oxysporum*, and JONES (17), who attributes stem end rot of tubers to the activity of this organism, although they may have dealt with "Mischarten." SHERBAKOFF (33) reports certain strains of species of ELEGANS (using the section as a morphological group) to be tuber rotters. He distinguishes between *Fusarium* spp. that are tuber rotters and such as are vascular element inhabitants.[1]

(2) EXPERIMENTAL INFECTION OF LIVING PLANTS

A series of experimental inoculations of healthy potato plants with *F. oxysporum* and *F. trichothecioides* were carried out, in an attempt to determine whether or not *F. trichothecioides* is unable to produce wilt, or whether the potato plant enjoys an effective or practical immunity rather than an absolute one. Even though Wollenweber (41) did not consider *F. trichothecioides* a wilt

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[1] After these experiments had been concluded and this paper written, a paper by CARPENTER (7) has appeared. This represents a wholly independent although simultaneously conducted piece of work. The results of CARPENTER make it quite probable that the observations made by the writer on a few strains of *F. oxysporum* are of quite general application, since he arrives at the same conclusions for numerous though different strains of *F. oxysporum*. His conclusions as to the method of attack by the fungus and the nature of the rot are practically identical with the writer's.

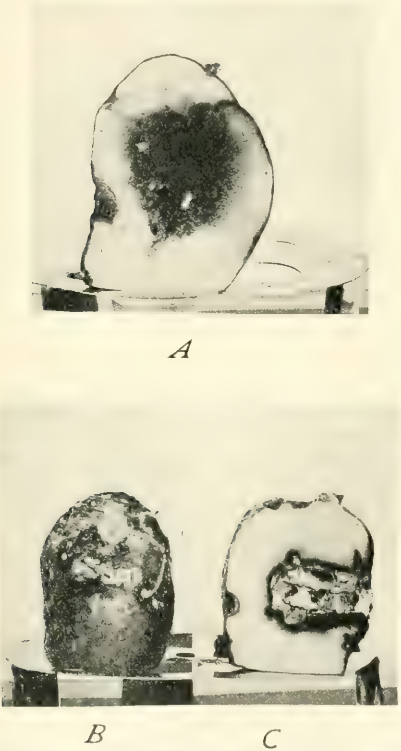

Fig. 1.—Tuber rot produced in laboratory with *Fusarium oxysporum*, and *F. trichothecioides*; *A*, soft rot produced by *F. oxysporum*, incubated at 20° C. for 17 days, Early Ohio variety; *B*, exterior of tuber rotted by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety; *C*, dry rot produced by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety.

producer in his 1913 paper, there is a reference in the 1912 paper by Jamieson and Wollenweber (**16**) to a wilt produced by *F. trichothecioides*. They referred to inoculation experiments, and report wilting in 12 days, "accompanied by a yellowing of the leaves and a discoloration of the tissue." The results of all of the writer's attempts of 1911–1912 to produce wilting of potato plants with *F. trichothecioides*, excepting one, were negative. During the past winter, however, it was noticed again and again that sprouts of tubers experimentally infected with this organism were dying. Microscopic and cultural studies left no doubt that this organism was responsible for the death of the sprouts.

Encouraged by these observations, the writer carried out some preliminary experiments on potato plants. Quartz was sterilized in 6 inch flower pots in the autoclave, and 8 plants that were about 10 cm. high were transplanted into these, the stems of some being smeared with rice infected with *F. trichothecioides*, and those of others with rice infected with *F. oxysporum*. The plants so inoculated and the controls were kept under bell jars. In three days the three plants smeared with *F. oxysporum* and two smeared with *F. trichothecioides* were dead, while the third one of the latter set and the controls remained healthy. The experiment was also conducted with potato plants growing in the open bench in the greenhouse, with similar results. The soil in this case was not sterilized.

The potato plants used in the following experiments were grown from sterilized tubers of the Early Ohio and Red Cobbler varieties in soil in 6 inch pots which had been thoroughly sterilized by heating in an autoclave for 4 hours on two consecutive days at 15 lb. pressure. The soil was watered with sterile water throughout the experiments.

On February 15, fifteen pots were planted with Early Ohio tubers and the soil of one set of 5 was infected with rice infected with *F. oxysporum*, of another with rice infected with *F. trichothecioides*, while the third set was left as a control. The controls came up in due time, while not a single one of the others came up. This experiment was repeated several times, but in no case was so striking a result obtained, although it often happened that some sprouts showed lesions, that some failed to come up, and that some were tardy in coming up. *Fusarium* spp. were isolated from such lesions. These lesions are identical in appearance with lesions found on potato stems and roots in the field which often are designated as "foot disease" and ascribed to the activity of *Rhizoctonia*. Late in May other series were started and the soil was infected with rice and spore suspensions. No infections resulted at all, even though the inoculum was derived from the same source as that used in earlier experiments.

On March 12, sprouts that were just breaking through the ground were uncovered and smeared with rice infected either with *F. oxysporum* or with *F. trichothecioides*, 6 sprouts being used in each set. The plants were wounded no more than was inevitable in removing the soil. The soil was then replaced. The soil in the controls was removed in the same way, but no inoculum was applied. The 12 sprouts to which inoculum had been applied were killed, while the controls remained healthy. There was no spreading of the disease to other sprouts, even where an abundance of inoculum was applied.

The affected sprouts reminded one forcibly of affected sprouts in potato fields in the spring. Here and there in the fields one finds sprouts that look sickly and small, which usually wilt and die or remain sickly and small. Upon examination of such sprouts, prominent brownish, watery lesions are found. At times such sprouts overcome the trouble and make a fair growth, at least until transpiration becomes excessive. These lesions also account for many of the "poor stands" or failures of potatoes to come up evenly. If one digs in where a sprout ought to have come up, one can often find a tuber that has sprouted, but whose sprouts have been cut off entirely by such lesions. Often lateral buds develop into branches on such decapitated sprouts, only to be cut off again. If such a tuber finally manages to get a shoot above the ground, the shoot is sickly and backward. In 1912, 1913, and 1914 the writer plated the inner tissue of many such sprouts and almost invariably obtained cultures of various *Fusarium* spp., although often associated with *Rhizoctonia* and bacteria. Infection experiments conducted with *Rhizoctonia* in 1912–1913 gave almost uniformly negative results. The writer was at first inclined to refer the major part of the potato troubles to the activity of this organism. Even though it is not the sole or even the main cause of Nebraska potato troubles it may play an important rôle. The work of APPEL (2), CORSAULT (8), DRAYTON (9), and MORSE and SCHAPOVALOV (26) gave results similar to those obtained by ROLFS (31, 32).

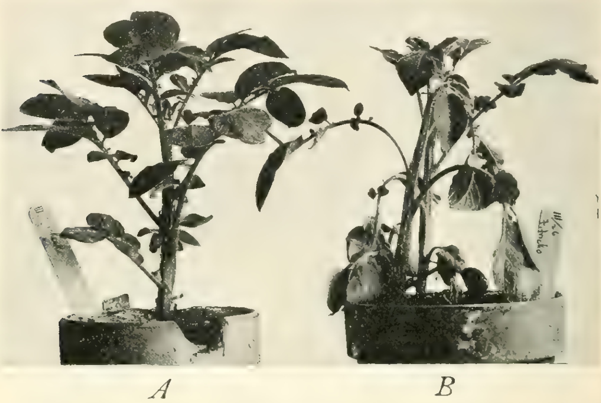

FIG. 2.—Wilt produced in laboratory with *Fusarium trichothecioides*, and control plant; *A*, control, Early Ohio variety; *B*, wilting and drying of leaves, 4 days after inoculation, Early Ohio variety.

On March 13, 24 plants grown in sterile soil were used in another experiment. These plants were about 10 cm. high at the time. The soil was removed from one shoot in each pot and the pots were arranged in 6 series. In series *A* the shoots were wounded and the wound smeared with *F. oxysporum* infected rice; in series *B* the sound stem was smeared with *F. oxysporum* infected rice; in series *C* the wounded shoots were smeared with *F. trichothecioides* infected rice; in series *D* the sound stems were smeared with *F. trichothecioides* infected rice; in series *E* no inoculum was applied to the wounded shoots; in series *F* the soil was merely removed and replaced (figs. 2 and 3).

On March 15 the following notes were taken. Series *A:* plant 1, slight curling of leaves; 2, apparently sound; 3, curling of leaves; 4, curling of leaves. Series *B:* plant 1, drooping leaves; 2, lower leaves drooping, upper leaves drying; 3, apparently sound; 4, apparently sound. Series *C:* plant 1, apparently sound; 2, some wilting; 3, some wilting; 4, some wilting. Series *D:* plants 1, 2, and 3 apparently sound; 4, wilting. By March 21 the plants infected with *F. oxysporum* showed a pronounced folding upward of leaves on the midrib, wilting and rolling on the margins of the leaves, the folding being most pronounced in the tips of the plants. The plants affected least showed discoloration on the margins, which at times was of a yellowish tint, at times purplish to violet. The leaves of plants most severely affected showed a yellowing and burning of the leaf margins. One plant, inoculated with *F. oxysporum*, developed a pronounced rosette, but overcame this later, growing into quite a normal plant (figs. 4 and 5). These symptoms remind one forcibly of certain symptoms of the leaf-roll disease which has received so much attention, and which has been made the subject

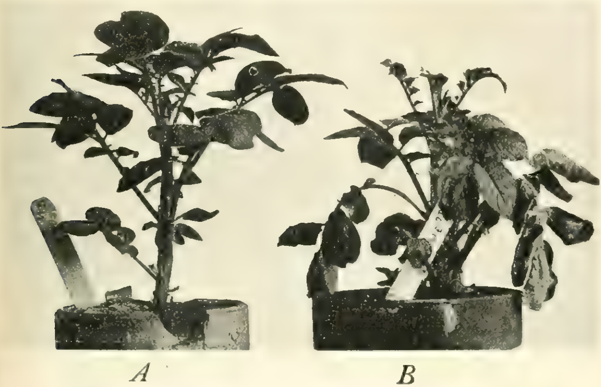

FIG. 3.—Wilt produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, wilting of lower leaves and curling of upper leaves, 4 days after inoculation, Early Ohio variety.

of thorough study by APPEL and his coworkers (**2**, **4**). Eventually the plants infected with *F. trichothecioides* showed much severer symptoms than those inoculated with *F. oxysporum* (fig. 6). Eight plants died in the former sets, and 3 in the latter. Plants infected with *F. trichothecioides* showed such severe and rapid burning and drying up of leaves that the typical wilting phenomena were scarcely realized. The vascular bundles were blackened and the blackening extended even into the petiole and the leaf veins. This rapid killing was at first strictly localized on that side of the plant to which the inoculum had been applied, even in the leaf, where the leaflets on one side of the midrib would be affected, and those on the other side not. Eventually in those cases in which killing of

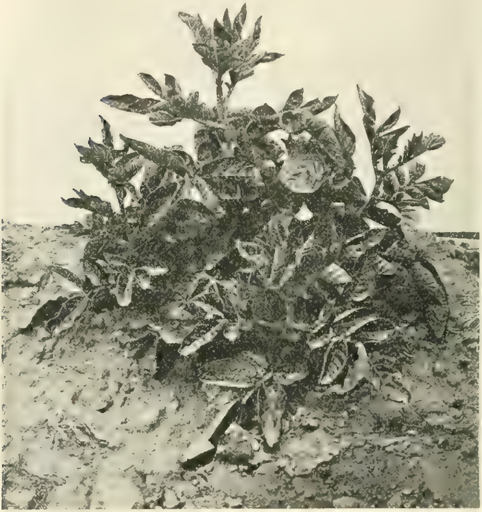

FIG. 4.—Leaf roll and rosette of field plant of the Pearl Variety; August 1912, at the U.S. Substation at Mitchell, Neb.

the whole plant took place, the fungus girdled the whole stem, while plants that were not girdled lived on, even though one side was entirely destroyed. There was little lateral and subsequent vertical spreading of the fungus from one vascular strand to the other. These experiments were repeated with 25 other plants and in most cases the same symptoms were observed. These symptoms have been repeatedly observed in the dry land areas of Nebraska, but have always been looked upon as cases of "sun scald," and and in previous experiments with wilting due to *F. trichothecioides* such cases were ignored.

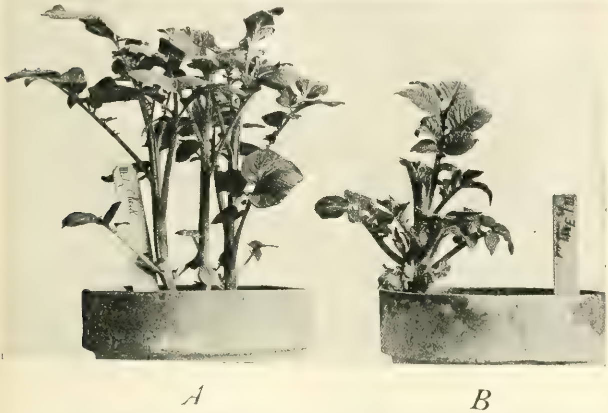

FIG. 5.—Rosette produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, rosetted plant, 10 days after inoculation, Early Ohio variety.

Plants grown in soil infected with *F. oxysporum* and *F. trichothecioides* showed severe lesions of root and stolons. Examination of roots affected with either organism showed that the cortical regions are first and most severely attacked, not only intercellularly, but also intracellularly, the cells being packed full with hyphae. In most cases the cortex could be sloughed off with exceeding ease. From the cortex the organisms invaded the stelar regions, where

F. oxysporum makes greater headway than the other and there causes a vascular mycosis more frequently, which accounts for its designation as a vascular parasite (figs. 7 and 8).

DISCUSSION.—If plants, experimentally inoculated, showed only light symptoms to begin with, most of them continued their growth with symptoms less severe than those shown in the field. If they showed severe symptoms early, these proved more severe and more rapidly fatal than those in the field. The organisms in the field work much more insidiously, attacking the roots of the plant slowly but progressively, and permitting the plant, except in extreme cases, to readjust for its water requirements. These readjustments manifest themselves in the curling and rolling phenomena (figs. 4 and 9).

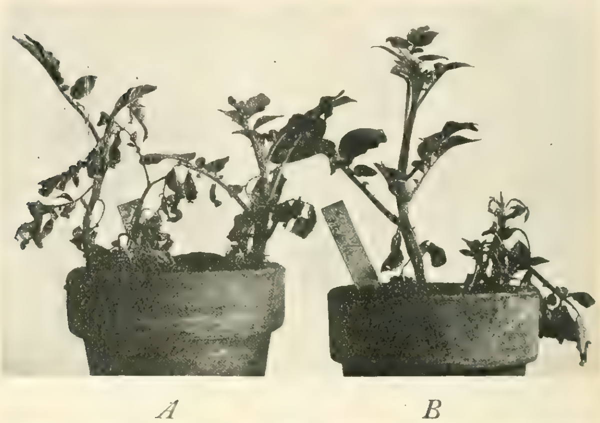

FIG. 6.—Wilt and death of potato plants produced in laboratory with *Fusarium trichothecioides*, 12 days after inoculation; Early Ohio variety; wilting is restricted to the side to which inoculum was applied.

Potato plants in the irrigated sections show this phenomenon nicely. As long as cultivation and irrigation are maintained, the plant develops new roots progressively higher up, and the infected plants get along fairly well, showing slight curling and wilting, although tuber development occurs. When in midsummer

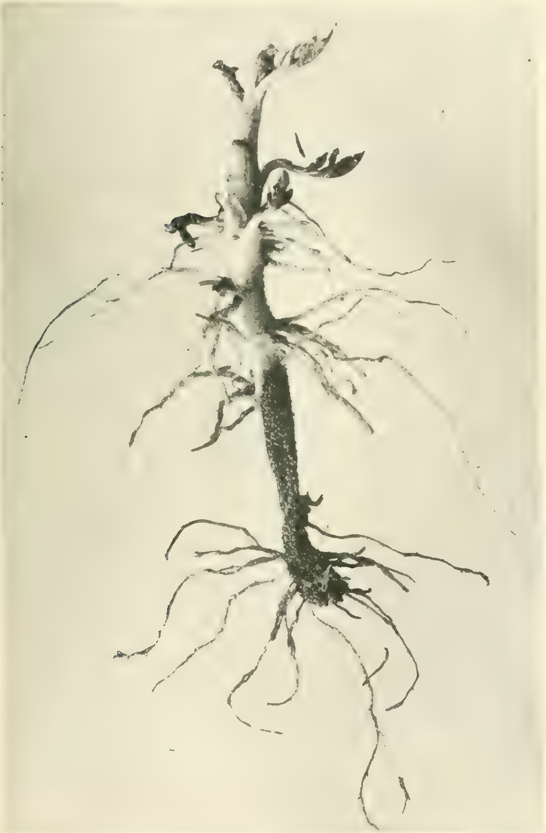

FIG. 7.—Lesions on stem and roots produced in laboratory with *Fusarium oxysporum*, 2 weeks after inoculation; Early Ohio variety.

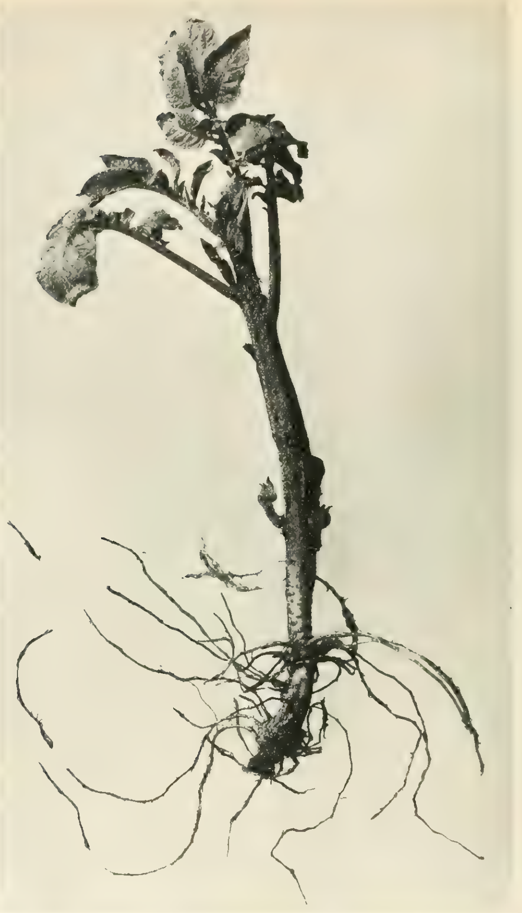

FIG. 8.—Root lesions produced in laboratory with *Fusarium trichothecioides*, curling and rolling of leaves, 2 weeks after inoculation; Early Ohio variety.

irrigation ceases and no more soil is heaped about the crown of the plant and transpiration requirements must be met by badly infected

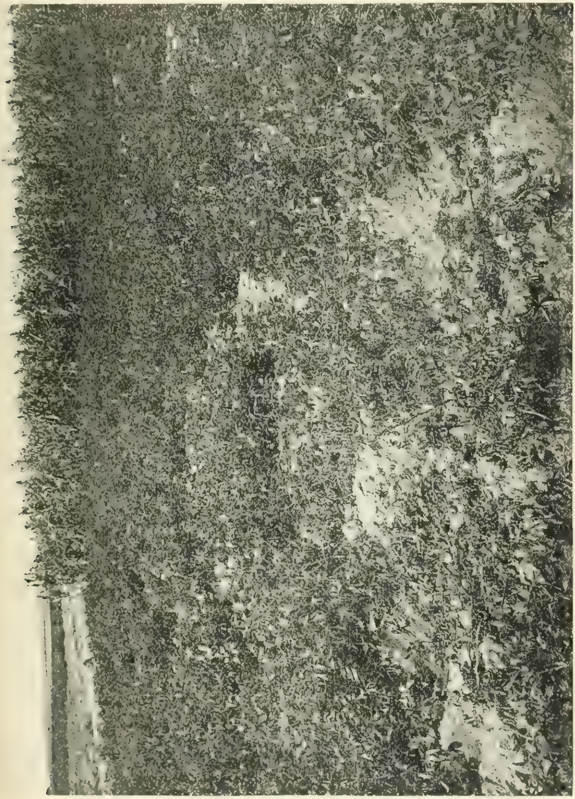

Fig. 9.—Potato wilt and little potatoes in a field of the Pearl variety, August 1912, at Mitchell, Neb.

lower roots and a few healthy upper ones without the possibility of developing new roots, the plant soon succumbs. In this way we get the exceedingly frequent phenomenon of large plants, usually with many small tubers, wilting down suddenly after the last irrigation. The frequent occurrence of aerial tubers, the prevalence of excessive numbers of small tubers, and the occurrence of few abnormally large tubers on such plants is also attributable to the insidious manner of attack. The organisms attack the stolons and main stem as well as the roots. Stolons with tubers in all stages of maturity can be found partially or completely cut off by lesions. As the balance between the photosynthetic and storage centers in such plants is disturbed, new stolons are developed nearer and nearer the surface and the stolons that are not attacked develop abnormally large tubers. Often the plant responds to this disturbance in the assimilation-storage balance by producing swellings of the aerial parts of the plant, the so-called aerial tubers. Many large plants can be pulled up with ease, because lesions make separation of the tops from the roots or even the basal portion of the stem easy. Such plants may show a comparatively sound main axis (fig. 10).

Infection carried over by the mother tuber, which is frequent, rarely permits the growth of stems more than 20 cm. high, and seldom allows the development of tubers. An early attack from without upon the main stem leads to equally disastrous results.

The wilts of the potato plant induced by *Fusarium* spp. have generally been considered vascular mycoses due to a clogging of the vascular elements. In fact, however, the symptoms are due to killing of the root system as much as to clogging of the vascular elements. It is true that members of the ELEGANS section, such as *F. oxysporum*, frequent the vascular elements, spreading in these rather than clogging them, but it is true also that they destroy roots in numbers. Again, even though some have referred to this disease as a root disease (SMITH and SWINGLE 35), it is stated that the fungus enters a root, then spreads to the stelar part, and from there enters other roots and stolons. Just as much damage is done by the persistent attack from without upon roots and stolons, as noted by MANNS (24).

In the course of these experiments several questions were raised. The soil in these experiments surely was more severely infected with the organisms than soil under field conditions can

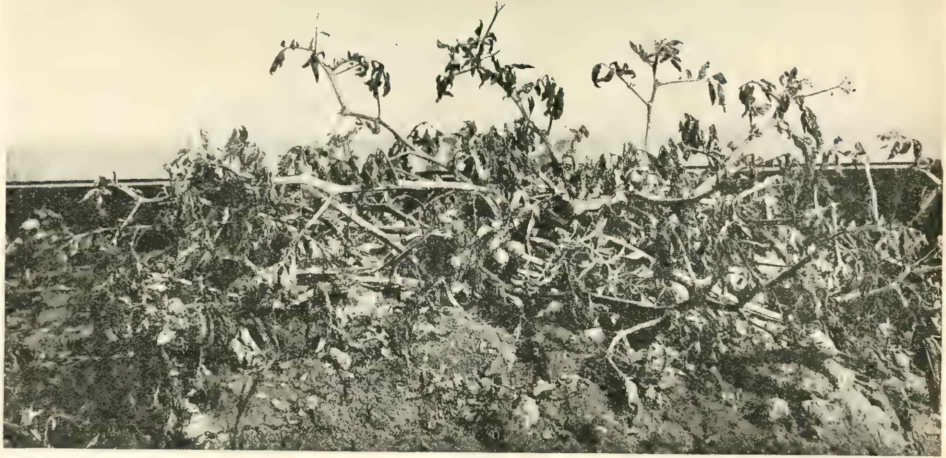

FIG. 10.—A plant of the field shown in fig. 9; after showing slight curling and discoloration of leaves and formation of aerial tubers in earlier part of summer this plant suddenly wilted down and died; such a plant as this usually bears a great number of small and a few abnormally large tubers which are infected in the stem end with the fungus that killed the mother plant.

be, yet there were many plants grown in such soil that showed no infection whatsoever. Less success in producing wilt was observed as the season progressed. It remains a question whether this is due to a loss in vitality or to a gain in resistance in the plants, due to a change in the soil, tubers, or the organism.

Whether the success in producing wilt with *F. trichothecioides* and the apparent waning of this power is due to a gain or regain of virulency and a subsequent loss again is also an unanswered question. SHERBAKOFF (33), working with *Fusarium* spp., got uniformly negative results so far as producing wilt is concerned, and concluded that the results were due to a loss of virulency of the cultures or to some other important factor that had escaped attention.

Summary

It is quite apparent that some of the strains of *F. oxysporum* can cause tuber rot; that they can destroy tubers entirely without the aid of other *Fusarium* spp. or bacteria; that at least one *Fusarium* of the DISCOLOR section (*F. trichothecioides*) can produce wilt of stem; and that the biological contrast drawn by WOLLENWEBER between the *Fusarium* spp. is not as sharp as one would infer from his paper. It is possible that these strains of *Fusarium* spp. are morphologically identical with those described by WOLLENWEBER, but physiologically unlike them. That this rule, if it exists, is not so rigid generally, however, is noted by SHERBAKOFF (33), who found that no correlation exists between morphological relationship and pathogenicity.

Although *F. oxysporum* is not absolutely unable to attack potato parenchyma, the potato tuber, in which usually only the xylem elements are invaded, enjoys an effective immunity from its attacks; and although *F. trichothecioides* can attack any subterranean part of the living potato plant, generally all parts excepting the mature tuber enjoy an effective immunity from its attacks.

The data given in the second part of this paper may furnish a partial explanation of these phenomena.

II. Ecology and physiology of the organisms

METHOD AND DATA

1. TEMPERATURE RELATIONS.—Observations of cultures grown at ordinary temperatures showed that there is a striking difference in the rate of growth of the two organisms. Potato cylinder, rice, liquid potato, and glucose media, and glucose and potato agar cultures all showed that *F. oxysporum* makes a considerably greater initial growth at temperatures above 20° C. than does *F. trichothecioides.* At temperatures in the vicinity of 10–15° C., however, *F. trichothecioides* makes the greater initial growth, although these temperatures lie below its optimum. The same difference was noted in cultures on neutral and acid potato agar. This point was also tested with cultures on sterile slabs of potato tubers kept in Petri dishes. At 25° C. *F. oxysporum* covered such slabs completely when *F. trichothecioides* barely had made a start, while at 12° C. the situation was reversed.

When 1 per cent liquid glucose media were inoculated with spore suspensions of *F. oxysporum*, visible growth was made in 16 hours; when *F. trichothecioides* was used, 30–42 hours elapsed before visible growth was made. This holds for temperatures above 20° C. The optimum temperature for *F. oxysporum* was about 30° C., and for *F. trichothecioides* about 20–22° C., both varying slightly with the medium used. The maximum for *F. oxysporum* lay between 38 and 40° C. The optima and maxima were higher for cultures in potato extract than for glucose media cultures. The writer has not been able to determine the minima accurately because of inadequate apparatus. HUMPHREY (15) gives 4° C. as the minimum growth temperature for a certain strain of *F. oxysporum.*

Potato agar cultures of *F. oxysporum* and *F. trichothecioides* could endure a temperature of 40° C. for 5 and for 20 hours respectively and remain viable. Exposure at 50° C. for 5 hours killed *F. trichothecioides*, but not *F. oxysporum;* while exposure for 20 hours killed both. Some *F. oxysporum* cultures survived 5 hours exposure at 57° C.

The growth relations were also checked up quantitatively. In these experiments, as well as in all the following ones, the method suggested by HASSELBRING (14) was followed. Erlenmeyer flasks of 200 cc. capacity were used with 50 cc. of solution per flask. The solutions in the flasks were autoclaved for 10 minutes at 7 lb. pressure, and then inoculated by means of sterile pipettes with a drop or two of spore suspension. The cultures were killed by adding 10 cc. of 10 per cent HCl to each flask. The cultures were then filtered off on tared Gooch crucibles prepared with asbestos, washed until acid free, and brought to constant weight in a Freas electric oven at 100° C., and the dry weight determined. It was found impossible at times to filter luxuriant cultures of *F. oxysporum* by this method, because of the tenacity with which this organism holds water. Consequently they were filtered on soft filter paper, transferred to tared Gooch crucibles, dried, and weighed. The other organism holds water with little tenacity and filters with ease.

In all of experiments given below the following stock mineral solution was used: 20 gm. NH_4NO_3; 10 gm. KH_2PO_4; 5 gm. $MgSO_4$ per 1000 cc. H_2O. When carbohydrates were employed,

TABLE I

DRY WEIGHT (IN MILLIGRAMS) AFTER 20 DAYS' GROWTH IN POTATO EXTRACT MEDIUM; ROOM TEMPERATURE

	FUSARIUM OXYSPORUM					
	Temperature					
	35°	30°	25°	12°	1°.11*	—1°.11*
Flask 1	40	55	63	64	62	86
Flask 2	47	78	68	66	....	....
Flask 3	61	86	80	68	....	....
Average	49	73	70	66	62	86

	FUSARIUM TRICHOTHECIOIDES					
	Temperature					
	35°	30°	25°	12°	1°.11*	—1°.11*
Flask 1	0	0	60	87	146	83
Flask 2	0	0	64	100	....	....
Flask 3	0	0	65	147	....	....
Average	0	0	63	111	146	83

*For 20 days (no growth), then at 25° C. for 25 days.

these were added at the rate of 10 gm. per liter. Potato extract medium was made up by extracting 500 gm. ground potato tuber with 500 cc. H_2O, and then adding 500 cc. of the foregoing solution to the extract.

A series of cultures (table II) was run at 12° C. and the amount of dry weight formed determined at 2 day intervals for 10 days. In this series the medium was at 12° C. at the time of inoculation.

TABLE II

DRY WEIGHT (IN MILLIGRAMS) IN POTATO EXTRACT MEDIUM; TEMPERATURE 12° C.

	FUSARIUM OXYSPORUM					FUSARIUM TRICHOTHECIOIDES				
	Number of days					Number of days				
	2	4	6	8	10	2	4	6	8	10
I	0.2	0.4	5.2	...	13.2	1.4	4.0	9.4	....	27.0
II	0.6	0.4	5.6	...	13.0	1.8	4.4	12.2	.....	44.6
Average	0.4	0.4	5.4	.....	13.1	1.6	4.2	10.8	.....	35.8

Table III shows the growth by day intervals made for 10 days when levulose was used as the carbon source. The solutions were at the temperatures indicated at the time of inoculation.

TABLE III

DRY WEIGHT (IN MILLIGRAMS) FORMED BY DAY INTERVALS

	FUSARIUM TRICHOTHECIOIDES AT 25° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I	0.2	0.6	4.2	12.8	29.0	19.2	25.4	19.4	34.0	43.6
II	0.2	2.4	18.0	31.6	32.4	34.2	27.2	41.9	47.7	50.8
Average	0.2	1.5	11.1	22.2	30.7	26.7	26.3	30.6	40.8	47.2

	FUSARIUM OXYSPORUM AT 25° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I	0.4	1.8	9.2	16.0	23.6	19.6	24.0	24.1	30.9	37.0
II	0.8	3.2	10.8	20.2	33.4	21.2	27.5	26.0	34.8	38.8
Average	0.6	2.5	10.0	22.6	28.5	20.4	25.7	25.0	32.8	37.9

TABLE III—*Continued*

	Fusarium oxysporum at 30° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I.	1.0	3.6	15.4	17.8	37.6	45.8	55.8	66.0	78.2	62.2
II.	8.9	5.2	17.6	19.0	40.2	77.8	62.6	69.6	78.6	66.6
Average.	4.9	4.4	16.5	18.4	38.9	61.8	59.2	67.8	78.4	64.4

	Fusarium trichothecioides at 25° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average...	4.4	19	67.4	......	......	......	......	262	......	......

	Fusarium oxysporum at 30° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average...	11.2	48	108.6	......	......	......	......	240.2	......	......

These tables show a tendency of *F. trichothecioides* to make a greater initial growth at low temperatures. At higher temperatures, however, unless above the optimum of *F. trichothecioides*, *F. oxysporum*, even though it made the greater initial growth, was soon overtaken and passed by *F. trichothecioides*. This was especially marked when dextrose and levulose were used as carbon source. This may be the result of a faster though more superficial feeding of *F. oxysporum*, which makes it unable to use materials as thoroughly as the other organism. This phenomenon is hardly a case of more rapid intoxication on the part of *F. oxysporum.*

The results obtained with artificial media were verified by infection experiments conducted with potato tubers kept at various temperatures. Tubers of the Red Cobbler variety were used. These were inoculated on April 1, and examined on May 27 (table IV). See figs. 11 and 12.

It should be noted here that *F. trichothecioides* when inoculated into a tuber can grow at 30° C., while it cannot do so in artificial media; and that *F. oxysporum* can survive a temperature of 1° C. in artificial media, but not in the tuber.

TABLE IV

CONDITION OF TUBERS AT CLOSE OF EXPERIMENT

Temperature	Fusarium oxysporum	Fusarium trichothecioides
30° C.	All completely rotted; sprouts killed	Slight rot in some
25° C.	All completely rotted; sprouts killed	All completely rotted; some sprouts killed
12° C.	All with very slight rot	All completely rotted
1° C.	No rot	All with slight rot
1° C. for two weeks, then 25° C. for two weeks	Slight rot in one tuber	All completely rotted
−1° C.	No rot	No rot
−1° C. for two weeks, then 25° C. for two weeks	No rot	All completely rotted

DISCUSSION.—These results may, in part at least, explain why *F. oxysporum*, even though it can attack parenchyma and rot tubers, usually is not found in rotted tubers, while *F. trichothecioides* is. The ability of the latter to make a faster initial growth at the temperatures which prevail in the soil about digging time and in well kept storage places is probably the determining factor in this phenomenon. The experiments with tubers showed that *F. trichothecioides* made a great increase in growth rate when transferred from a low to a higher temperature.

These temperature relations may also explain in part the fact that we usually find *F. oxysporum* producing wilt under field conditions, and lend support to the observations made by ORTON (**27**), who reports potato wilt induced by *Fusarium* spp. to be preeminently a warm climate disease. *F. trichothecioides* can produce wilt, but the temperature conditions in the soil are such as to favor *F. oxysporum*, the maximum temperature of the former being the optimum of the latter. HUMPHREY (**15**), working in Washington on the tomato wilt induced by *F. oxysporum*, came to the conclusion that temperature differences in various parts of the state were determining factors for the appearance and non-appearance and severity of the disease.

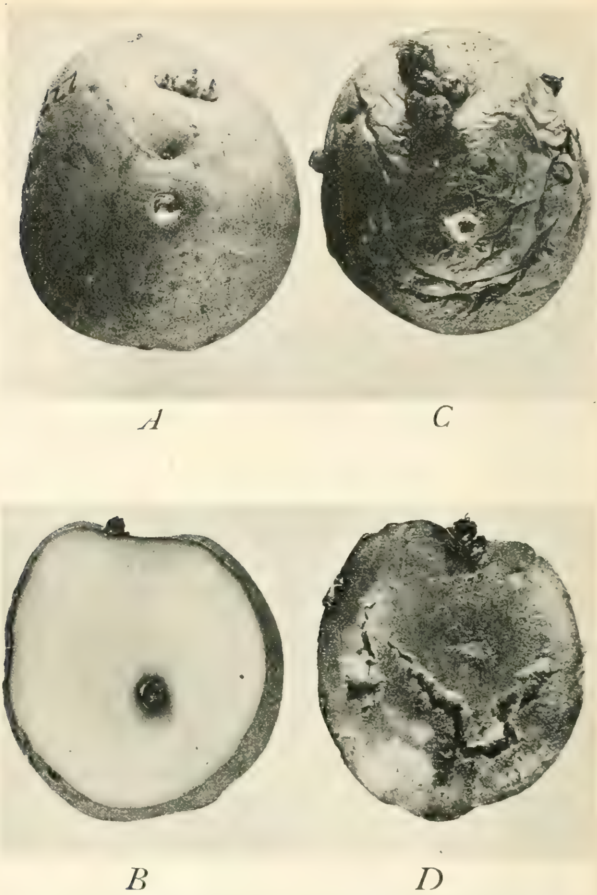

FIG. 11.—Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium oxysporum; A, B*, external and sectional view of same tuber, incubated for 30 days 12° C.; *C, D*, external and sectional view of same tuber, incubated for 30 days at 25° C.

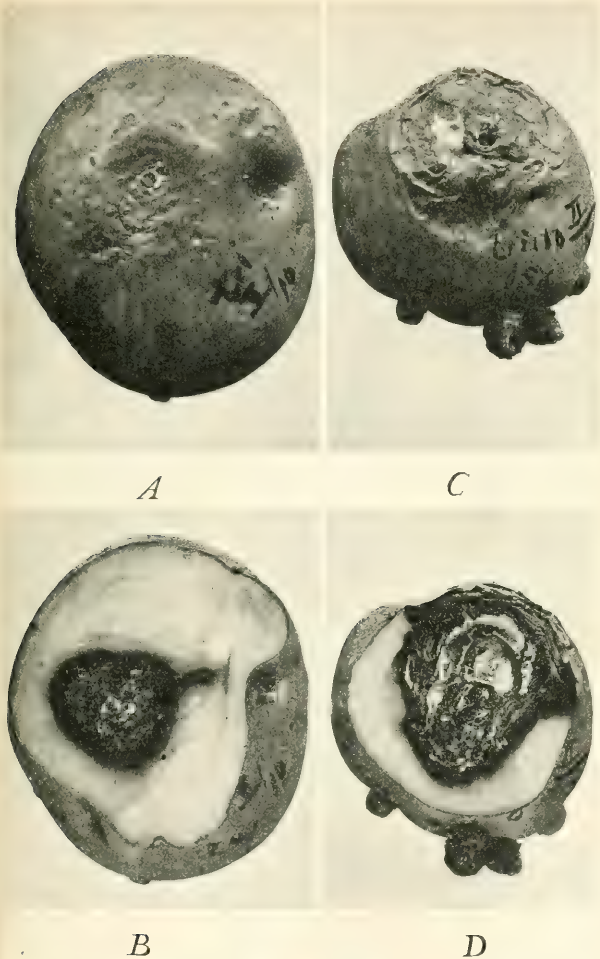

FIG. 12.—Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium trichothecioides;* *A*, *B*, external and sectional view of same tuber, incubated for 30 days at 25° C.; *C*, *D*, external and sectional view of same tuber, incubated for 30 days at 12° C.

2. GROWTH HABIT.—It was observed in nearly all cultures that *F. oxysporum* not only made a greater initial growth at ordinary temperatures, but that it was at all temperatures much more of a surface grower than *F. trichothecioides*, making a superficial spreading growth, rather than the penetrating restricted intensive growth of the latter. Early sporulation was associated with the restricted growth habit of the latter (fig. 13). These habits were especially clearly marked on solid substrata, but even in liquid media *F. oxysporum* made a much less compact growth than the other species. It may be that the more spreading and extensive growth habit of *F. oxysporum* at all temperatures and its more rapid initial growth at temperatures above 10–15° C. are associated with a greater oxygen requirement than that possessed by *F. trichothecioides*. This would explain in part the frequenting of intercellular spaces and xylem elements by the former, and its consequent greater efficiency in causing vascular mycosis and wilt, as well as its tendency to cause bundle discoloration. The xylem elements of the stem end are undoubtedly infected while the tuber is yet in the soil, where temperature conditions are such as to favor the growth of *F. oxysporum*. Storage temperatures check the growth of this organism and the cells bordering the infected vascular elements shut the infected area off by suberizing their walls. Cultural experiments and microscopical studies show that cork is not absolutely impenetrable to these organisms, although it provides under normal conditions an effective barrier to the progress of both of these species. Because of the slower growth of *F. trichothecioides* at higher temperatures, the potato plant undoubtedly has a much better opportunity to guard itself by cork formation against this organism than against the other.

3. THE CARBON SOURCES OF THE TWO ORGANISMS.—A difference in the metabolic requirements of two organisms, a difference in their ability to utilize various substances, or a difference in their ability to tolerate the presence of substances may be factors of critical importance in determining which of the two will attack a given tissue or a given plant. These factors may determine also the modes of attack of an organism upon a tissue or a plant. Thus an organism that can digest pectinaceous material and not cellulose

would have to destroy a tissue whose walls are mainly cellulose by intercellular activity, while one that could digest cellulose might destroy this tissue by a primary cell invasion. Again, a greater ability on the part of an organism to digest suberin, other things

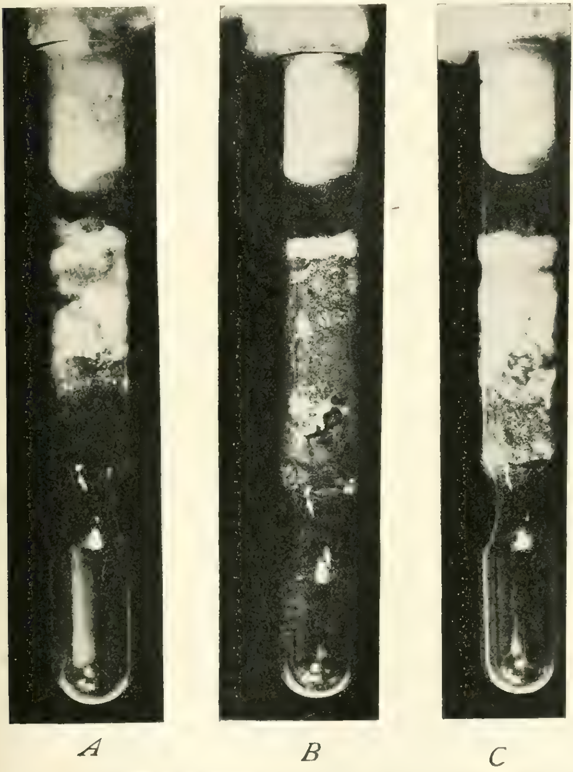

FIG. 13.—*Fusarium trichothecioides* and *F. oxysporum* on sterile potato cylinders; *A*, *C*, cylinders inoculated with *F. oxysporum*, incubated for 2 days at 25° C.; *B*, cylinder inoculated with *F. trichothecioides*, incubated for 2 days at 25° C.

being equal, would render it a much more formidable enemy of the potato plant than an organism without this ability, or possessing it to a less degree. This problem was attacked by making a study of the carbon sources of the organisms. The data reported here are only a beginning of this phase of the problem.

Fifty cc. of nutrient solution were measured quantitatively into 200 cc. Erlenmeyer flasks with a pipette. The flasks were then plugged with cotton, covered with tinfoil, and autoclaved. After cooling, 0.5 gm. of carbohydrate material was transferred quantitatively into each flask, and the flasks covered again with tinfoil and sterilized in a Freas oven by heating at 85° C. for one hour every 12 hours, for 6 consecutive days. The solutions were then incubated at 25° C. for 48 hours, so as to allow any contaminations present to appear. Low sterilization temperature was used to reduce hydrolysis of carbohydrates to a minimum.

The dry weight determinations were made by the methods outlined above. It was found advisable to kill two cultures of each set after 6 days, for the striking differences in rate of growth between the two organisms that were observed during the first 48-120 hours were obliterated by prolonged growth. The other 3 cultures were killed after 12 days' growth. The dry weight values do not show the differences in habit and rate of growth in the cultures as strikingly as they appeared to the eye. In many cases a visible growth was not determinable as dry weight. This is readily appreciated when we consider that moisture determinations indicated that the dry weight varied between 10 and 20 per cent of the wet weight.

In the controls, consisting of the plain mineral medium without carbon material, *F. oxysporum* made a weighable growth in 12 days, though not in 6 days, while *F. trichothecioides* made no weighable growth even after 12 days. Another important observation was made. In no case was it necessary to reinoculate with *F. oxysporum*, while many *F. trichothecioides* inoculations failed. The latter undoubtedly is the slower starter and much more poorly equipped for sure and quick infection than the former.

The figures in tables V–VIII represent milligrams of dry weight of material formed, except in those cases in which per cent is written. In such cases (cork, cellulose, and hemicellulose) the figures represent the percentage of decrease in dry weight of material. The last weighing in these cases unavoidably included the dry weight of fungus material formed, so that the figures are higher than they ought to be. The differences in weight in these cases give only comparative values of the amounts of material respired by the organisms. The filter paper used was the best Swedish paper, and the cork was obtained by skinning steamed potatoes, scrubbing the skin thoroughly, boiling it for 48 hours in distilled water, extracting for 48 hours in ether, and then boiling again with water. All figures represent averages, the composition of these figures being shown in tables VI and VII. In many cases there was a fair coincidence of the values, while in others a great disparity appeared. The averages probably would more nearly approximate the true value if a greater number of figures were available.

TABLE V

DRY WEIGHT (IN MILLIGRAMS) FORMED IN 6 AND 12 DAYS BY *Fusarium trichothecioides* AND *F. oxysporum*

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Ethyl alcohol	...	1.2	15.0	18.5
Glycerine	12.0	20.5	17.0	92.0
Mannit	108.5	136.3	109.5	112.0
Arabinose	5.2	35.0	78.7	43.0
Glucose	11.5	42.3	43.5	44.0
Mannose	2.0	52.0	49.0	62.0
Galactose	28.8	43.3	39.0	73.0
Fructose	56.5	81.0	74.5	77.6
Saccharose	9.5	45.6	36.5	35.6
Maltose	13.0	61.0	35.5	50.3
Lactose	1.3	20.0	4.0	21.0
Raffinose	12.4	47.3	44.5	54.6
Potato starch	...	45.0 per cent	81.0	103.0
Wheat starch	22.6 per cent	42.1 per cent	54.3 per cent	127.0
Corn starch	19.7 per cent	33.4 per cent	37.9 per cent	67.2 per cent
Soluble starch	45.2 per cent	49.0 per cent	110.5	210.3
Dextrine	4.4	37.3	42.0	56.3
Inulin	63.6	123.0	102.0	90.0
Gum arabic	1.5	18.6	37.0	58.3
Gum tragacanth	67.5 per cent	14.1 per cent	27.1 per cent	25.7
Hemicellulose	10.1 per cent	7.3 per cent	10.1 per cent	6.7 per cent
Cellulose	4.0 per cent	1.0 per cent	4.25 per cent	6.7 per cent
Cork	10.1 per cent	5.9 per cent	12.8 per cent	3.9 per cent
No carbon source	...	...	...	0.5

Table V shows that qualitatively the two organisms behave alike in their ability to use all the carbon compounds tested. Quantitatively there is considerable difference, both as to rate of consumption and total growth after 12 days. *F. oxysporum* in general shows the greater speed of growth and greater growth after twelve days. In some cases *F. trichothecioides* shows the greater growth after 12 days.

TABLE VI

DRY WEIGHT (IN MILLIGRAMS) FORMED WHEN EACH FLASK RECEIVED 0.5 GM. OF MANNIT; CONCENTRATION 1 PER CENT

NUMBER OF FLASK	FUSARIUM OXYSPORUM		FUSARIUM TRICHOTHECIOIDES	
	6 days	12 days	6 days	12 days
I	93	....	107	....
II	126	....	110	....
III	....	108	....	130
IV	....	112	....	135
V	....	116	....	141
Average	109.5	112	108.5	136.3

TABLE VII

HEMICELLULOSE (IN GM.) USED IN 12 DAYS

NO. OF FLASK	FUSARIUM OXYSPORUM			FUSARIUM TRICHOTHECIOIDES		
	6 days			6 days		
	Grams of material	Decrease in weight	Percentage of decrease	Grams of material	Decrease in weight	Percentage of decrease
I	0.5055	0.0455	9.0	0.5213	0.0513	9.8
II	0.5154	0.0584	11.3	0.4964	0.0524	10.5
Average	....	....	10.1	....	....	10.1
	12 days			12 days		
III	0.5185	0.0355	6.1	0.501	0.028	5.5
IV	0.5192	0.0382	7.3	0.5058	0.0488	9.4
Average	....	....	6.7	....	....	7.3

Table VIII gives the dry weight formed by the organisms when carbon acids were furnished as carbon sources. N/100 solutions were used, excepting for asparagin and asparagenic acid, whose solubility permitted only N/200 solutions. In the case of the higher fatty acids and oils, the material was weighed out as though N/100 solutions were being prepared. With these no weighings of the material formed were attempted, but merely differences in luxuriance of growth were recorded. To those acids which showed no growth with N/100 solutions, 5 cc. of 10 per cent glucose solution was added, making the sugar concentration 1 per cent, so as to determine whether the acid was merely non-usable, or whether it was toxic. Since it was found that some were toxic at N/100 concentration, lower concentrations were made up also. The results are given in table IX.

TABLE VIII

DRY WEIGHT (IN MILLIGRAMS) FORMED WITH THE FOLLOWING CARBON COMPOUNDS AS CARBON SOURCES

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Formic acid N/100	None	None	None	None
+5 cc. 10 per cent glucose solution	“	“	“	85.5
Acetic acid N/100	“	“	1.2	10.6
+5 cc. 10 per cent glucose solution	“	106	None	None
Proprionic acid N/100	“	None	“	“
+5 cc. 10 per cent glucose solution	“	“	“	“
Butyric acid N/100	“	“	“	“
+5 cc. 10 per cent glucose solution	“	“	“	“
Glycocollic acid N/100	2.5	7.3	20.0	8.6
Lactic acid N/100	0.3	0.6	8.0	23.0
Oxalic acid N/100	Non-weighable	Non-weighable	Non-weighable	Non-weighable
Succinic acid N/100	Non-weighable	Non-weighable	8.5	9.3
Malic acid N/100	Non-weighable	Non-weighable	4.5	8.0
Tartaric acid N/100	1.0	0.93	6.0	4.3
Citric acid N/100	2.6	6.6	8.0	6.6
Aspartic acid N/200	1.6	....	5.5	6.5
Asparagin N/200	3.5	3.8	5.5	5.3
Tannic acid 1 per cent	1.0	31.0	0.2	42.0
Tannic acid 0.5 per cent	1.6	61.0	1.6	41.0
+5 cc. 10 per cent glucose	3.5	32.0	5.0	47.0
Control 0.5 gm. levulose	....	82.0	....	116.0

TABLE VIII—*Continued*

	FUSARIUM TRICHOTHECIOIDES	FUSARIUM OXYSPORUM
	12 days	12 days
Palmitic acid I	No growth	Good growth
“ “ II	Slight “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+5 cc. 10 per cent dextrose, V	Excellent “	“ “
Stearic acid, I	Slight “	“ “
“ “ II	No “	“ “
“ “ II	No “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+ 5 cc. 10 per cent dextrose, V	Excellent “	“ “
Oleic acid, I	No “	“ “
“ “ II	Poor “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+5 cc. 10 per cent dextrose, V	Slight “	“ “
Palm oil, I	No “	No “
“ “ II	“ “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+5 cc. 10 per cent dextrose, V	“ “	“ “
Olive oil, I	Fair “	Fair “
“ “ II	“ “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “

A marked difference was found in the ability of the two organisms to use the fatty acids, *F. trichothecioides* being much more restricted in its ability. The experiments with alcohol and the acids also showed that the former organism was much more readily poisoned and inhibited in its growth. It was found that *F. oxysporum* grew well in 1 per cent ethyl alcohol, and that *F. trichothecioides* made no growth. The solution was then diluted one-half, whereupon *F. trichothecioides* made a good growth. This was clearly a case of inhibition. The growth of *F. trichothecioides* was inhibited by N/100 acetic acid, as can be seen by the fact that it grew in N/125 concentration and that it grew in N/100 when glucose was added, while *F. oxysporum* grew well in N/100 acetic acid. N/100 formic acid was toxic to *F. trichothecioides*, while it merely inhibited growth with the other organism. The latter grew in N/125 formic acid, while *F. trichothecioides* did not grow in N/500 solution. N/100 proprionic acid was toxic to both, while both grew in N/250 solution. N/250 butyric acid was toxic to *F. trichothecioides*, while *F. oxysporum* grew in it.

TABLE IX

DRY WEIGHT (IN MILLIGRAMS) FORMED IN 12 DAYS IN VARIOUS CONCENTRATIONS OF ACID

Concentration	Fusarium oxysporum				Fusarium trichothecioides			
	N/1000	N/500	N/250	N/125	N/1000	N/500	N/250	N/125
Formic acid								
I	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	None	None	None
II	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	"	"	"
Average	...	...	...	...	...	...	...	...
Acetic acid								
I	...	...	...	...	0.8	1.2	3.2	5.8
II	...	...	...	...	0.8	2	3.4	6.6
Average	...	...	...	...	0.8	1.6	3.3	6.2
Proprionic acid								
I	1.4	2.0	5.0	None	1.4	2.6	4.0	None
II	2.6	2.4	5.8	"	1.2	3.4	6.6	"
Average	2.0	2.2	5.4	...	1.3	3.0	5.3	...
Butyric acid								
I	4.8	7.6	10.4	None	2.4	5.0	None	None
II	5.4	9.0	13.8	"	3.6	5.6	"	"
Average	5.1	8.3	12.1	...	3.0	5.3	...	...
Oxalic acid								
I	0.4	0.4	0.4	1.2	0.8	Non-weighable	Non-weighable	Non-weighable
II	0.8	1.2	1.2	1.2	0.8	Non-weighable	Non-weighable	0.2
Average	0.6	0.8	0.8	1.2	0.8	...	...	0.1

A set of experiments was run also in which solanin in various percentages was added to glucose media. Because of the high cost of solanin, only 1 cc. solution was used in each test. The results are given in table X.

These differences, that is, greater versatility in the use of carbon sources, greater resistance to inhibition, and intoxication, may well play an important rôle in determining the difference in behavior of these two organisms.

TABLE X

DRY WEIGHT (IN MILLIGRAMS) FORMED BY *Fusarium oxysporum* AND *F. trichothecioides* IN 6 DAYS

	Percentage solanin				
	1	2	0.25	0.226	0
Fusarium oxysporum....	10.6	8.1	13.2	10	13.8
Fusarium trichothecioides	2.5	2.4	7.8	....	9.3

DISCUSSION.—The versatility of these organisms in using various carbon sources in their metabolism is of great interest. This almost omnivorous ability to use carbon compounds, including the simplest fatty acid, the highly oxidized fatty acids, the long carbon chain acids, the alcohols, mono- and poly-hydric, glycerin and fats, the mono-, di-, and poly-saccharides, including the dextrines, starches, hemicelluloses, and true celluloses, assigns to them an important rôle in the carbon cycle, and at the same time must help render them the formidable and destructive enemies of the root crops that they are.

The methods suggested by APPEL (**2**), namely, rigid inspection of potato fields, immediate destruction of all plants that show the slightest symptoms, quarantining of non-certified seed stock, alone give promise of keeping these troubles in check. Disinfection of storage cellars and of potatoes when put into storage, together with storage at proper temperature, will help combat these diseases, especially the dry rot induced by *F. trichothecioides*.

Conclusions

1. *Fusarium tubcrivorum* Wilcox and Link is the same as *Fusarium trichothecioides* Woll.

2. Both *Fusarium oxysporum* and *F. trichothecioides* can produce both tuber rot and wilt of the potato plant.

3. The wilt is induced by destruction of the root system and by clogging of the xylem elements in the stem, and is, in mild cases, marked by such symptoms as discoloration of leaves, curling and rolling of leaves, and production of aerial tubers.

4. Under field and storage conditions *Fusarium oxysporum* is more probably responsible for wilt than is *F. trichothecioides*, and the latter more responsible for tuber rotting.

5. The optimum and maximum temperatures of *Fusarium oxysporum* are higher than those of *F. trichothecioides*. *F. trichothecioides*, however, grows well at 8-10° C., while *F. oxysporum* does not. These facts may explain in part the fact that *F. oxysporum* produces more wilt than *F. trichothecioides*, and that the latter causes more tuber rot.

6. *Fusarium oxysporum* has a more rapid, superficial, and spreading habit of growth than has *F. trichothecioides*. This may be associated with a greater oxygen requirement for *F. oxysporum*, and may account for the frequenting of xylem elements by this fungus.

7. Both organisms possess a striking ability to use the most diverse carbon materials as carbon sources in their metabolism. *Fusarium oxysporum* has a greater range in its ability, and can utilize the materials more readily, although not so completely as does *F. trichothecioides*.

8. *Fusarium oxysporum* is less subject to inhibition in growth and intoxication than is *F. trichothecioides*.

9. Solanin is not toxic to either organism, although it seems to inhibit somewhat the growth of *Fusarium trichothecioides*.

The writer acknowledges his indebtedness to Dr. E. MEAD WILCOX and to Dr. WILLIAM CROCKER. They not only made this research possible, but they gave freely of advice and criticism, and lent encouragement by their interest in the progress of the investigation.

AGRICULTURAL EXPERIMENT STATION
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A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO

By GEO. K. LINK

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A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **219**

George K. K. Link

WITH THIRTEEN FIGURES

There is little doubt among phytopathologists that members of the genus *Fusarium* play an important role in producing diseased conditions in many plants, both wild and cultivated. According to Wollenweber (**41**), *Fusarium* spp. produce wilt in members of the following families: Liliaseae, Bromeliaceae, Musaceae, Solanaceae, Convolvulaceae, Leguminosae, Malvaceae, Linaceae, Cucurbitaceae. Cruciferae, Compositae, Araliaceae, Caryophyllaceae, and Pedaliaceae.

History

The genus *Fusarium* was established by Link (**20, 21**) in 1809, and *Fusarium* species were reported on rotted and ring-discolored tubers by Martius in 1842, Hartig in 1846, and Schacht in 1856. Pizzigoni (**29**) and Wehmer (**38, 39**) demonstrated by experimental inoculation that *Fusarium* species can bring about tuber rot. They referred to the *Fusarium* in question as *F. solani*. Others, however, among them Frank (**11, 12**), repeating their work, obtained negative results so far as *Fusarium* species were concerned; while De Bary (**6**) and many others regarded the *Fusarium* spp. as nothing more than obligate saprophytes.

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The credit of first demonstrating experimentally the relation of *Fusarium* spp. to certain plant wilts belongs to SMITH (**34**), who found a *Fusarium* responsible for watermelon wilt. SMITH and SWINGLE (**35**) reported a potato wilt and tuber rot which they considered due to a *Fusarium* which they called *F. oxysporum*. They considered this organism identical with *F. solani* of PIZZIGONI and WEHMER, and used the oldest name available, *F. oxysporum* (SCHLECHTENDAHL, 1824); however, they reported no experimental inoculations. PETHYBRIDGE and BOWERS (**28**) reported a dry rot due to *F. solani*, and LONGMAN (**22**) also reported a dry rot due to a *Fusarium*.

Many pathologists and mycologists entertained considerable doubt as to the parasitic nature of *Fusarium* spp., while others were quite convinced of their parasitic nature. SORAUER (**36**) was quite positive in his decision, while MASSEE (**25**) wavered. LINDAU (**18**) remained skeptical and referred to the *F. oxysporum* of SMITH and SWINGLE as a "Mischart." DUGGAR (**10**) was quite positive in his decision. Much of this difference of opinion undoubtedly was due to the confusion that prevailed as to the status of *F. solani*, *F. oxysporum*, and the genus in general, since no basis for extended morphological study of the genus had been established, and even the genus itself had not been sharply defined. MASSEE (**25**) considered *F. solani* to be the conidial form of *Nectria solani;* while REINKE and BERTHOLDT (**30**) considered it the conidial form of *Hypomyces solani*. LOUNSBURY (**23**) tried to arbitrate the matter by suggesting that *F. solani* and *F. oxysporum* are one and the conidial stage of *Nectria solani*. APPEL and WOLLENWEBER (**5**) published a monograph in which they defined the genus and brought some order into the chaos of species. Among other radical changes they dropped *F. oxysporum* and established *F. orthoceras* in its place.

MANNS (**24**) demonstrated by experimental inoculation that a *Fusarium*, which he designated (following SMITH and SWINGLE) as *F. oxysporum*, could produce tuber rot and wilt. He made no morphological studies, however, and undoubtedly had not had access to APPEL and WOLLENWEBER's monograph.

JAMIESON and WOLLENWEBER (**16**) published an account of a dry rot of tubers induced by a *Fusarium* which they described as a new species (*F. trichothecioides* Wr.). They refer to it as "a wound parasite capable of destroying potato tubers" and say "this disease is clearly differentiated from the wilt and dry rot ascribed by Smith and Swingle to *F. oxysporum*."

Later, the writer (**19**) submitted his studies of a dry rot occurring among Nebraska potatoes as a thesis to the Graduate Faculty of the University of Nebraska. The work was done at the request of Dr. E. Mead Wilcox, and consisted in part of a study of the morphology of a *Fusarium* that had been isolated from dry rotted tubers in 1908 by Miss Venus W. Pool from potatoes that farmers had sent in from throughout the state during the season 1907-1908. Miss Pool established the causal relation of this *Fusarium* to the dry rot by experimental infection, and named the organism in manuscript *F. pulverulentum*, because of its powdery habit of growth. Both field and laboratory work were carried on for several years, and it was found that this organism caused primarily a dry rot of the tuber, and that it was not the *F. oxysporum* of Smith and Swingle, a culture of *F. oxysporum* having been furnished the laboratory for comparative work through the courtesy of Dr. Smith. The results were to have been published in 1911, and the organism was to be named *F. pulverulentum*, but upon the appearance of Appel and Wollenweber's monograph Dr. Wilcox proposed to the writer that he reinvestigate the organism along the lines suggested by these authors. This was especially desirable since *F. oxysporum* had been dropped and several new species established. Not only was this carried out, but the whole etiology was gone over again and all of Miss Pool's results verified. It was found that Appel and Wollenweber (**5**) had not described the species, and consequently it was described as *F. tuberivorum* Wilcox and Link (**40**). It was so named because of the apparent restriction of its activity to tubers.

A comparison of this paper and the paper of Jamieson and Wollenweber (**16**) made it seem quite likely that both were dealing with the same organism. The organism was isolated in the Washington laboratories from potatoes sent in from Washington, Nebraska, and other states in 1910, and Wollenweber upon his arrival in the laboratory, using his monograph as the basis, described it as a new species. He told the writer in 1913 that he felt convinced that we had described one and the same thing. Comparative studies made by the writer during the past year verify this point of view, and since the Nebraska publication by WILCOX, LINK, and POOL (**40**) did not appear in print until 1913, the name *F. trichothecioides* should be adopted.

WOLLENWEBER (**41, 42**) published a further paper in which he categorized the *Fusarium* spp. very sharply, dividing the genus into sections on the basis of physiological (that is, pathogenicity) and morphological (that is, conidia and chlamydospores) characters. *F. oxysporum* was again established and taken as the representative of the section ELEGANS, which comprises vascular parasites; and *F. trichothecioides* was put into the section DISCOLOR, which comprises parenchyma destroyers. He distinguished sharply between these and also between the vascular ring-discoloring *Fusarium* species of section ELEGANS and the tuber-rotting *Fusarium* species of sections DISCOLOR, GIBBOSUM, MARTIELLA, etc.

Referring to the papers by SMITH and SWINGLE (**35**), MANNS (**24**), and others, particularly to that by MANNS, he writes: "They do not separate fusarioses causing tuber rot from those causing both the wilt diseases of the plant and ring discoloration of the tuber, so that the reader might conclude that both wilt disease and tuber rot are caused by the same organism." Referring to his own experiments, he writes: "It also brings out the striking fact that the fungus, a typical xylem inhabitant, does not entirely destroy the tuber without the help of tuber rot *Fusarium* or bacteria," and "the fact that *F. oxysporum* causes the wilt of growing potato plants and only uses the xylem of the stem end of tubers for over-wintering, without producing a rot of the parenchyma, leads to interesting comparisons with the following 4 species which are able to destroy the tuber entirely from artificial wounds, namely, *F. coeruleum* (Lib.), *F. trichothecioides* Wr.," etc., and finally "the fact that the latter (*F. oxysporum*) cannot produce a tuber rot gives a biological contrast to the wound parasites of the tuber, and the fact that they cause the wilt disease of the growing plant presents a contrast to the saprophytes."

Problem and method of attack

In the spring and summer of 1914 the writer discussed the *Fusarium* situation as outlined by WOLLENWEBER with Dr. E. M. WILCOX and Dr. WILLIAM CROCKER. The former suggested that the whole situation ought to be gone over, and the latter that it would be of interest to search for the physiological basis of this alleged biological contrast. It is clear that, if the strict categories of WOLLENWEBER exist, then potato parenchyma must possess either an absolute or an effective immunity toward *Fusarium* spp. of the ELEGANS section, and that *Fusarium* spp. of the DISCOLOR section are either absolutely or practically unable to produce vascular mycoses or wilts.

The purpose of this research was twofold: (1) to determine whether such a sharp biological contrast exists; and (2) to determine what is the physiological basis for such a contrast. Experimental infections of potato plants and tubers were used for the first phase of the problem. It was clear that the second phase might involve a great many considerations, such as the structural, compositional, and metabolic nature of both host and parasite, as well as the relation of environmental factors to these. The important role played by the structural and compositional peculiarities of the potato and the influence of external factors upon these is well illustrated by the studies of APPEL and KREITZ (**1, 3**) on the efficacy of the cork layer in checking bacterial invasions of the tuber. Considerations of time and equipment limitations made it obligatory that the scope of the work be limited to a study of a few representative strains of the groups.

The writer is under obligation to the Departments of Agricultural Chemistry, Horticulture, and Experimental Agronomy of the University of Nebraska Experiment Station for the use of materials and equipment; to Miss ETHEL BEATY for help in much of the laborious routine; to Dr. FLORENCE A. MCCORMICK for valuable help in the anatomical and microtechnical phases of the problem; and to Mr. R. A. DAWSON for help in preparing the photographs.

The writer decided to work with *F. oxysporum* as representative of the vascular parasite section (ELEGANS), and with

F. trichothecioides or *F. tuberivorum* as representative of the parenchyma-invading section (DISCOLOR). Since it was desirable that the identity of the organisms be well established, the writer asked Mr. W. A. ORTON, in whose laboratories Dr. WOLLENWEBER had carried out his recent work, for cultures of the organisms. It was impossible to get cultures which had been authenticated by Dr. WOLLENWEBER, since he had gone to war, but through the courtesy of Mr. ORTON, Mr. CARPENTER (Dr. WOLLENWEBER'S assistant) furnished a strain of *F. trichothecioides* (no. 41, 1916) and a strain of *F. oxysporum* (no. 3345A). The other strains of *F. trichothecioides* used had been isolated by the writer in 1911, and were described as *F. tuberivorum*. Several strains of *F. oxysporum* isolated from Nebraska potatoes were also used.

Pure cultures of these organisms were maintained on sterilized rice in plugged Erlenmeyer flasks, and these were used as a point of departure for all the work recorded.

I. Infection experiments

(1) EXPERIMENTAL INFECTION OF TUBERS

Tubers of the Early Ohio and Red Cobbler varieties were used in these experiments. Only sound tubers were selected, and these were thoroughly cleansed and sterilized before infection. At first they were sterilized by the formaldehyde gas method recommended by WOLLENWEBER (**41**). Several difficulties were encountered in using this method. It was found very difficult to remove the last traces of the gas without contaminating the chamber, and the tubers often showed the characteristic formaldehyde vapor injuries that have been discussed in bulletins of the New York Experiment Station (**13**, **37**). Consequently, the writer abandoned the first method and sterilized tubers by immersing in 1:1000 $HgCl_2$ solution for 1.5 hours. Inoculation was carried out by removing a piece of the cortex with a sterile cork borer, placing an infected grain of rice into the hole, and then replacing the piece of tuber tissue. The wound was then sealed with sterile grafting wax and the tuber placed into sterile chambers. This proved an efficient and convenient way of carrying out the great number of experimental inoculations made.

The first inoculations were made in December 1914. The cut ends of 20 Early Ohio tubers were wetted with spore suspensions of *F. trichothecioides* and 5 tubers were kept as controls. Four inoculated and one control potato were kept in each compartment at a temperature of 25° C. in an almost saturated atmosphere. After 4 weeks all of the inoculated tubers were in advanced stages of rot.

On January 31, 3 potatoes were inoculated according to the second method with *F. oxysporum*, and 3 with *F. trichothecioides*, and kept at 20° C. until February 17. Two of the former set were slightly rotted and one totally, while the entire latter set was rotted severely. The controls showed no rot (fig. 1).

On January 15 another series was started which was kept at a temperature ranging from 15-20° C. until February 15. Six sets of 3 tubers each were started and each set was kept in a separate sterile chamber, 2 tubers of each set being inoculated by smearing cut surfaces with agar grown inoculum. Sets I, II, and III were inoculated with *F. oxysporum*, and sets IV, V, and VI with *F. trichothecioides*. In set I, one inoculated tuber was rotted, while the other and the control were sound; in set II, one was deeply rotted and the others sound; in set III, one was deeply rotted and the others sound; in set IV, two were rotted and the control sound; in set V, two tubers were rotted slightly and the control sound; and in set VI, two tubers were rotted and the control sound.

F. oxysporum and *F. trichothecioides* were re-isolated from these rotted tubers by placing tissue cut from such tubers on plated glucose agar. Nothing other than the organism with which the tuber had been inoculated developed. Inoculum from these plates was used in infecting tubers again with the same results.

Since these results were at variance with the statements of Wollenweber the experiments were repeated with hundreds of tubers, and the results were verified.

Discussion.—Tubers inoculated with *F. oxysporum* did not develop the ring discoloration that is considered characteristic of the activity of *F. oxysporum*, but a general rot of the whole tuber. Generally, however, this was not a dry rot, but a rot that resembles more the soft rots of bacterial origin, although it is not accompanied by the offensive odors of bacterial rots, producing a blackening and softening of tissues which extends a considerable distance beyond the actual site of the organism. This was demonstrated microscopically and culturally. At times, however, especially in cold, dry conditions, a dry rot as typical as that produced by *F. trichothecioides* was produced. *F. trichothecioides* invariably produced a dry rot with only a very limited darkened zone extending beyond the destroyed zone, made up of large cavities and a mixture of disintegrated. dry, shrivelled tissue and fungus tissue. No darkening extended beyond the actual site of the fungus and no softening of tissue occurred. Microscopic examination revealed the fact that *F. trichothecioides* attacked the tissue intracellularly and destroyed each cell completely before it proceeded to the neighboring cell, while *F. oxysporum* attacked the tissue intercellularly at first, and then attacked the cells intracellularly, but not until the tissue had been blackened and disorganized. In this way a softened tissue without cavities was produced. These rots produced experimentally with pure cultures of *F. oxysporum* lend support to the observations and conclusions of Smith and Swingle (**35**), Manns (**24**), who report the occasional appearance of black specks in the parenchyma of tubers infected with *F. oxysporum*, and Jones (**17**), who attributes stem end rot of tubers to the activity of this organism, although they may have dealt with "Mischarten." Sherbakoff (**33**) reports certain strains of species of Elegans (using the section as a morphological group) to be tuber rotters. He distinguishes between *Fusarium* spp. that are tuber rotters and such as are vascular element inhabitants.[1]

(2) EXPERIMENTAL INFECTION OF LIVING PLANTS

A series of experimental inoculations of healthy potato plants with *F. oxysporum* and *F. trichothecioides* were carried out, in an attempt to determine whether or not *F. trichothecioides* is unable to produce wilt, or whether the potato plant enjoys an effective or practical immunity rather than an absolute one. Even though WOLLENWEBER (**41**) did not consider *F. trichothecioides* a wilt

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[1] After these experiments had been concluded and this paper written, a paper by Carpenter (7) has appeared. This represents a wholly independent although simultaneously conducted piece of work. The results of Carpenter make it quite probable that the observations made by the writer on a few strains of *F. oxysporum* are of quite general application, since he arrives at the same conclusions for numerous though different strains of *F. oxysporum*. His conclusions as to the method of attack by the fungus and the nature of the rot are practically identical with the writer's.

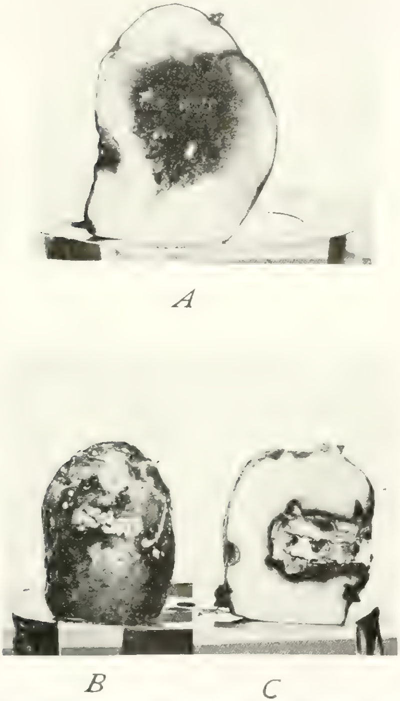

FIG. 1. Tuber rot produced in laboratory with *Fusarium oxysporum*, and *F. trichothecioides*. *A*, soft rot produced by *F. oxysporum*, incubated at 20° C. for 17 days, Early Ohio variety; *B*, exterior of tuber rotted by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety; *C*, dry rot produced by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety.

producer in his 1913 paper, there is a reference in the 1912 paper by JAMIESON and WOLLENWEBER (**16**) to a wilt produced by *F. trichothecioides.* They referred to inoculation experiments, and report wilting in 12 days, "accompanied by a yellowing of the leaves and a discoloration of the tissues." The results of all of the writer's attempts of 1911-1912 to produce wilting of potato plants with *F. trichothecioides,* excepting one, were negative. During the past winter, however, it was noticed again and again that sprouts of tubers experimentally infected with this organism were dying. Microscopic and cultural studies left no doubt that this organism was responsible for the death of the sprouts.

Encouraged by these observations, the writer carried out some preliminary experiments on potato plants. Quartz was sterilized in 6 inch flower pots in the autoclave, and 8 plants that were about 10 cm. high were transplanted into these, the stems of some being smeared with rice infected with *F. trichothecioides*, and those of others with rice infected with *F. oxysporum.* The plants so inoculated and the controls were kept under bell jars. In three days the three plants smeared with *F. oxysporum* and two smeared with *F. trichothecioides* were dead, while the third one of the latter set and the controls remained healthy. The experiment was also conducted with potato plants growing in the open bench in the greenhouse, with similar results. The soil in this case was not sterilized.

The potato plants used in the following experiments were grown from sterilized tubers of the Early Ohio and Red Cobbler varieties in soil in 6 inch pots which had been thoroughly sterilized by heating in an autoclave for 4 hours on two consecutive days at 15 lb. pressure. The soil was watered with sterile water throughout the experiments.

On February 15, fifteen pots were planted with Early Ohio tubers and the soil of one set of 5 was infected with rice infected with *F. oxysporum*, of another with rice infected with *F. trichothecioides*, while the third set was left as a control. The controls came up in due time, while not a single one of the others came up. This experiment was repeated several times, but in no case was so striking a result obtained, although it often happened that some sprouts showed lesions, that some failed to come up, and that some were tardy in coming up. *Fusarium* spp. were isolated from such lesions. These lesions are identical in appearance with lesions found on potato stems and roots in the field which often are designated as "foot disease" and ascribed to the activity of *Rhizoctonia.* Late in May other series were started and the soil was infected with rice and spore suspensions. No infections resulted at all, even though the inoculum was derived from the same source as that used in earlier experiments.

On March 12, sprouts that were just breaking through the ground were uncovered and smeared with rice infected either with *F. oxysporum* or with *F. trichothecioides*, 6 sprouts being used in each set. The plants were wounded no more than was inevitable in removing the soil. The soil was then replaced. The soil in the controls was removed in the same way, but no inoculum was applied. The 12 sprouts to which inoculum had been applied were killed, while the controls remained healthy. There was no spreading of the disease to other sprouts, even where an abundance of inoculum was applied.

The affected sprouts reminded one forcibly of affected sprouts in potato fields in the spring. Here and there in the fields one finds sprouts that look sickly and small, which usually wilt and die or remain sickly and small. Upon examination of such sprouts, prominent brownish, watery lesions are found. At times such sprouts overcome the trouble and make a fair growth, at least until transpiration becomes excessive. These lesions also account for many of the "poor stands" or failures of potatoes to come up evenly. If one digs in where a sprout ought to have come up, one can often find a tuber that has sprouted, but whose sprouts have been cut off entirely by such lesions. Often lateral buds develop into branches on such decapitated sprouts, only to be cut off again. If such a tuber finally manages to get a shoot above the ground, the shoot is sickly and backward. In 1912, 1913, and 1914 the writer plated the inner tissue of many such sprouts and almost invariably obtained cultures of various *Fusarium* spp., although often associated with *Rhizoctonia* and bacteria. Infection experiments conducted with *Rhizoctonia* in 1912-1913 gave almost uniformly negative results. The writer was at first inclined to refer the major part of the potato troubles to the activity of this organism. Even though it is not the sole or even the main cause of Nebraska potato troubles it may play an important role. The work of APPEL (**2**), CORSAULT (**8**), DRAYTON (**9**), and MORSE and SCHAPOVALOV (**26**) gave results similar to those obtained by ROLFS (**31, 32**).

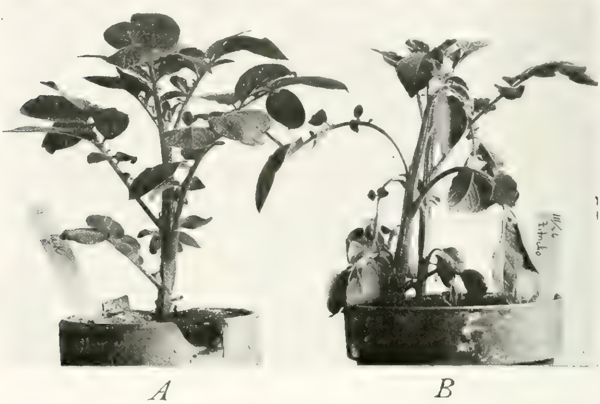

FIG. 2.—Wilt produced in laboratory with *Fusarium trichothecioides*, and control plant; *A*, control, Early Ohio variety; *B*, wilting and drying of leaves, 4 days after inoculation, Early Ohio variety.

On March 13, 24 plants grown in sterile soil were used in another experiment. These plants were about 10 cm. high at the time. The soil was removed from one shoot in each pot and the pots were arranged in 6 series. In series *A* the shoots were wounded and the wound smeared with *F. oxysporum* infected rice; in series *B* the sound stem was smeared with *F. oxysporum* infected rice; in series *C* the wounded shoots were smeared with *F. trichothecioides* infected rice; in series *D* the sound stems were smeared with *F. trichothecioides* infected rice; in series *E* no inoculum was applied to the wounded shoots; in series *F* the soil was merely removed and replaced (figs. 2 and 3).

On March 15 the following notes were taken. Series *A:* plant 1, slight curling of leaves; 2, apparently sound; 3, curling of leaves; 4, curling of leaves. Series *B:* plant 1, drooping leaves; 2, lower leaves drooping, upper leaves drying; 3, apparently sound; 4, apparently sound. Series *C:* plant 1, apparently sound; 2, some wilting; 3, some wilting; 4, some wilting. Series *D:* plants 1, 2, and 3, apparently sound; 4, wilting. By March 21 the plants infected with *F. oxysporum* showed a pronounced folding upward of leaves on the midrib, wilting and rolling on the margins of the leaves, the folding being most pronounced in the tips of the plants. The plants affected least showed discoloration on the margins, which at times was of a yellowish tint, at times purplish to violet. The leaves of plants most severely affected showed a yellowing and burning of the leaf margins. One plant, inoculated with *F. oxysporum*, developed a pronounced rosette, but overcame this later, growing into quite a normal plant (figs. 4 and 5). These symptoms remind one forcibly of certain symptoms of the leaf-roll disease which has received so much attention, and which has been made the

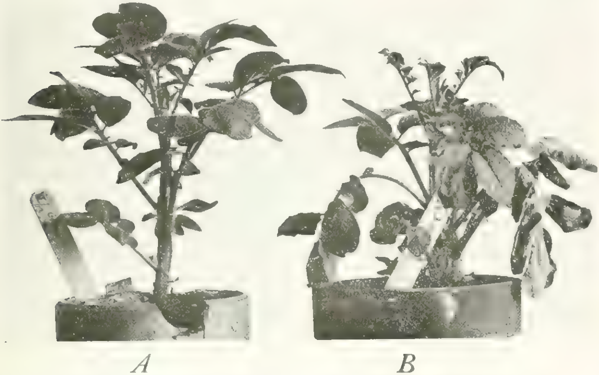

FIG. 3.—Wilt produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, wilting of lower leaves and curling of upper leaves, 4 days after inoculation, Early Ohio variety.

subject of thorough study by APPEL and his coworkers (**2**, **4**). Eventually the plants infected with *F. trichothecioides* showed much severer symptoms than those inoculated with *F. oxysporum* (fig. 6). Eight plants died in the former sets, and 3 in the latter. Plants infected with *F. trichothecioides* showed such severe and rapid burning and drying up of leaves that the typical wilting phenomena were scarcely realized. The vascular bundles were blackened and the blackening extended even into the petiole and the leaf veins. This rapid killing was at first strictly localized on that side of the plant to which the inoculum had been applied, even in the leaf, where the leaflets on one side of the midrib would be affected, and those on the other side not. Eventually in those cases in which killing of

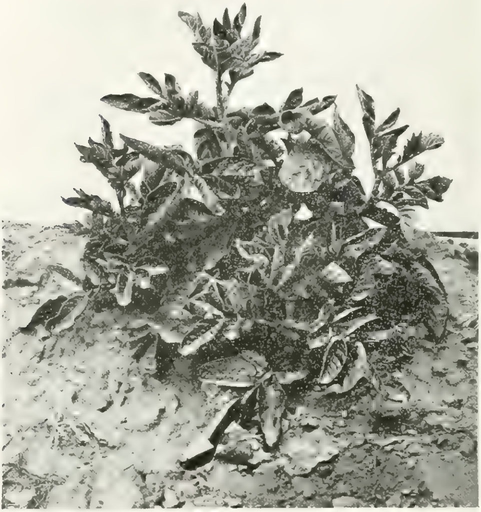

FIG. 4.—Leaf roll and rosette of field plant of the Pearl variety; August 1912, at the U. S. Substation at Mitchell, Neb.

the whole plant took place, the fungus girdled the whole stem, while plants that were not girdled lived on, even though one side was entirely destroyed. There was little lateral and subsequent vertical spreading of the fungus from one vascular strand to the other. These experiments were repeated with 25 other plants and in most cases the same symptoms were observed. These symptoms have been repeatedly observed in the dry land areas of Nebraska, but have always been looked upon as cases of "sun scald," and in previous experiments with wilting due to *F. trichothecioides* such cases were ignored.

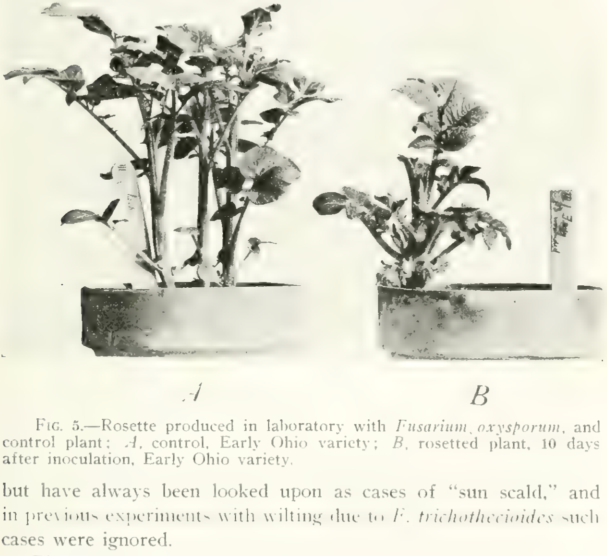

FIG. 5.—Rosette produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, rosetted plant, 10 days after inoculation, Early Ohio variety.

Plants grown in soil infected with *F. oxysporum* and *F. trichothecioides* showed severe lesions of roots and stolons. Examination of roots affected with either organism showed that the cortical regions are first and most severely attacked, not only intercellularly, but also intracellularly, the cells being packed full with hyphae. In most cases the cortex could be sloughed off with exceeding ease From the cortex the organisms invaded the stelar regions, where

F. oxysporum makes greater headway than the other and there causes a vascular mycosis more frequently, which accounts for its designation as a vascular parasite (figs. 7 and 8).

Discussion.—If plants, experimentally inoculated, showed only light symptoms to begin with, most of them continued their growth with symptoms less severe than those shown in the field. If they showed severe symptoms early, these proved more severe and

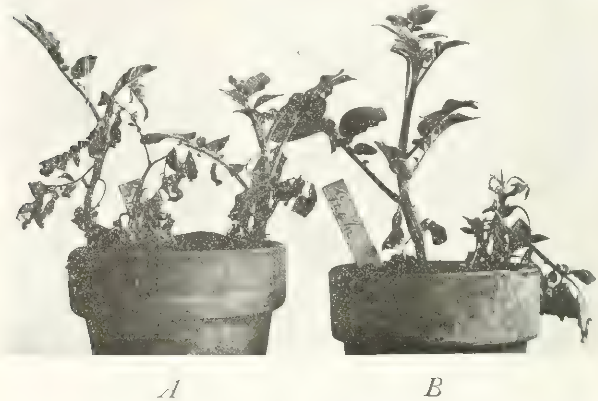

Fig. 6.—Wilt and death of potato plants produced in laboratory with *Fusarium trichothecioides*, 12 days after inoculation; Early Ohio variety; wilting is restricted to the side to which inoculum was applied.

more rapidly fatal than those in the field. The organisms in the field work much more insidiously, attacking the roots of the plant slowly but progressively, and permitting the plant, except in extreme cases, to readjust for its water requirements. These readjustments manifest themselves in the curling and rolling phenomena (figs. 4 and 9).

Potato plants in the irrigated sections show this phenomenon nicely. As long as cultivation and irrigation are maintained, the plant develops new roots progressively higher up, and the infected plants get along fairly well, showing slight curling and wilting, although tuber development occurs. When in midsummer

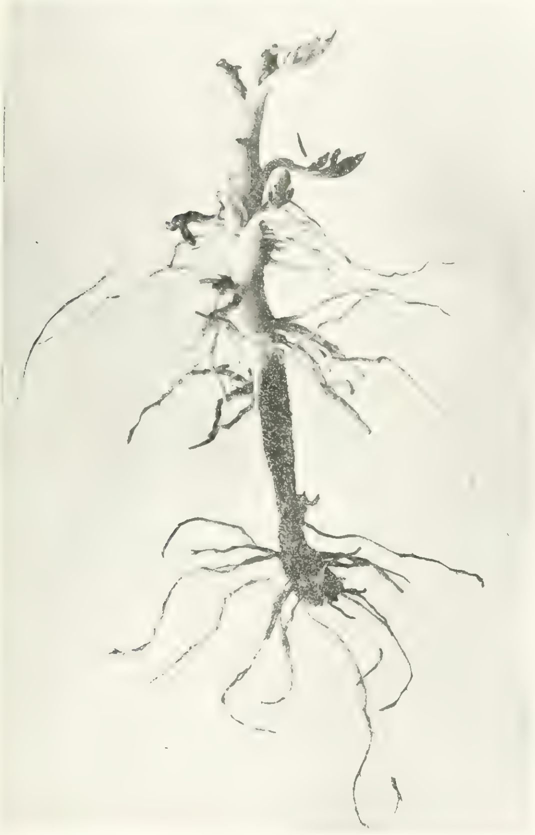

Fig. 7.—Lesions on stems and roots produced in laboratory with *Fusarium oxysporum*, 2 weeks after inoculation; Early Ohio variety.

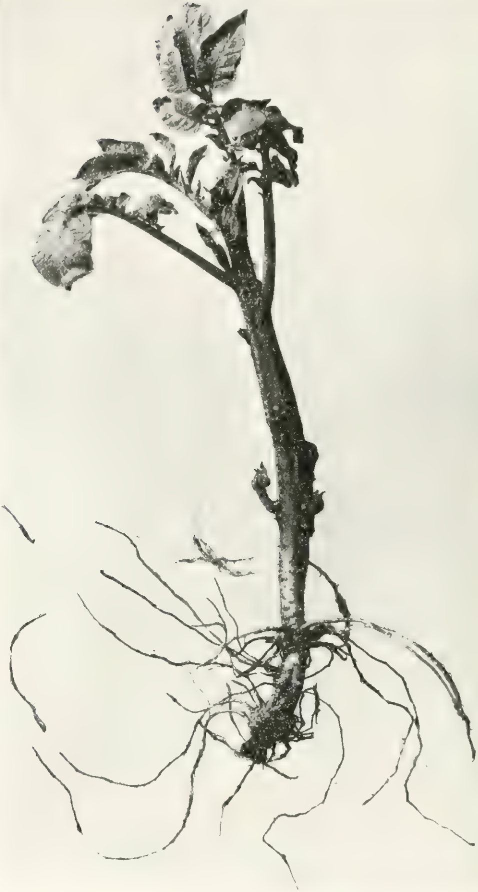

FIG. 8.—Root lesions produced in laboratory with *Fusarium trichothecioides*, curling and rolling of leaves, 2 weeks after inoculation; Early Ohio variety.

irrigation ceases and no more soil is heaped about the crown of the plant and transpiration requirements must be met by badly infected lower roots and a few healthy upper ones without the possibility of developing new roots, the plant soon succumbs. In this way we

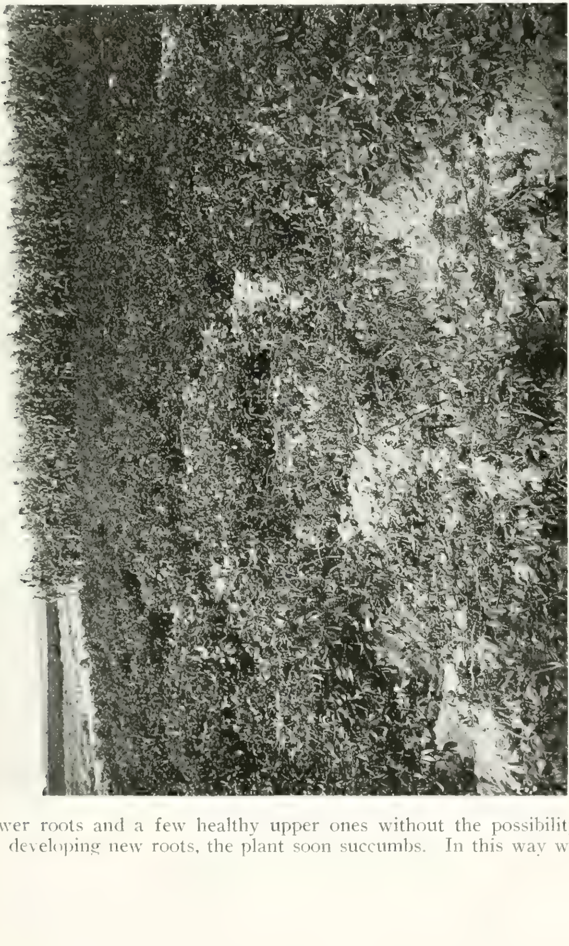

Fig. 9.—Potato wilt and little potatoes in a field of the Pearl variety, August 1912, at Mitchell, Neb.

get the exceedingly frequent phenomenon of large plants, usually with many small tubers, wilting down suddenly after the last irrigation. The frequent occurrence of aerial tubers, the prevalence of excessive numbers of small tubers, and the occurrence of few abnormally large tubers on such plants is also attributable to the insidious manner of attack. The organisms attack the stolons and main stem as well as the roots. Stolons with tubers in all stages of maturity can be found partially or completely cut off by lesions. As the balance between the photosynthetic and storage centers in such plants is disturbed, new stolons are developed nearer and nearer the surface and the stolons that are not attacked develop abnormally large tubers. Often the plant responds to this disturbance in the assimilation-storage balance by producing swellings of the aerial parts of the plant, the so-called aerial tubers. Many large plants can be pulled up with ease, because lesions make separation of the tops from the roots or even the basal portion of the stem easy. Such plants may show a comparatively sound main axis (fig. 10).

Infection carried over by the mother tuber, which is frequent, rarely permits the growth of stems more than 20 cm. high, and seldom allows the development of tubers. An early attack from without upon the main stem leads to equally disastrous results.

The wilts of the potato plant induced by *Fusarium* spp. have generally been considered vascular mycoses due to a clogging of the vascular elements. In fact, however, the symptoms are due to killing of the root system as much as to clogging of the vascular elements. It is true that members of the ELEGANS section, such as *F. oxysporum*, frequent the vascular elements, spreading in these rather than clogging them, but it is true also that they destroy roots in numbers. Again, even though some have referred to this disease as a root disease (SMITH and SWINGLE **35**), it is stated that the fungus enters a root, then spreads to the stelar part, and from there enters other roots and stolons. Just as much damage is done by the persistent attack from without upon roots and stolons, as noted by MANNS (**24**).

In the course of these experiments several questions were raised. The soil in these experiments surely was more severely

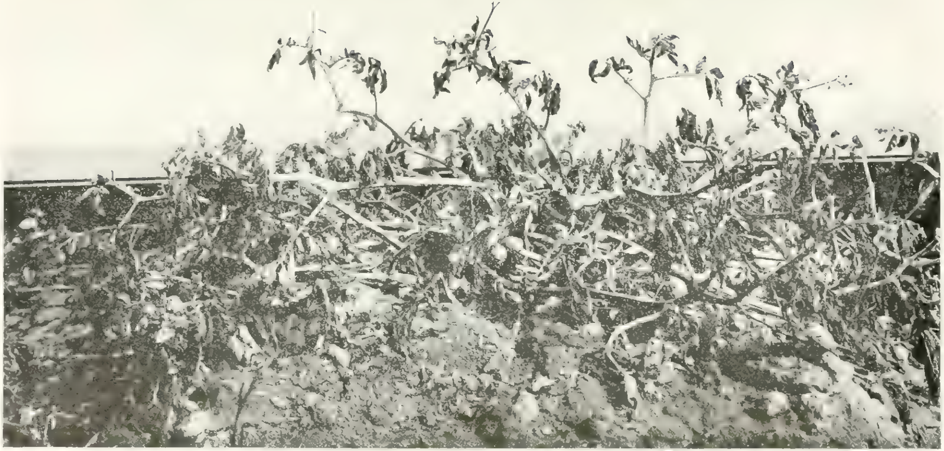

Fig. 10.—A plant of the field shown in fig. 9; after showing slight curling and discoloration of leaves and formation of aerial tubers in earlier part of summer this plant suddenly wilted down and died; such a plant as this usually bears a great number of small and a few abnormally large tubers which are infected in the stem end with the fungus that killed the mother plant.

infected with the organisms than soil under field conditions can be, yet there were many plants grown in such soil that showed no infection whatsoever. Less success in producing wilt was observed as the season progressed. It remains a question whether this is due to a loss in vitality of the organism or to a gain in resistance in the plants, due to a change in the soil, tubers, or the organism.

Whether the success in producing wilt with *F. trichothecioides* and the apparent waning of this power is due to a gain or regain of virulency and a subsequent loss again is also an unanswered question. SHERBAKOFF (**33**), working with *Fusarium* spp., got uniformly negative results so far as producing wilt is concerned, and concluded that the results were due to a loss of virulency of the cultures or to some other important factor that had escaped attention.

Summary

It is quite apparent that some of the strains of *F. oxysporum* can cause tuber rot; that they can destroy tubers entirely without the aid of other *Fusarium* spp. or bacteria; that at least one *Fusarium* of the DISCOLOR section (*F. trichothecioides*) can produce wilt of stem; and that the biological contrast drawn by WOLLENWEBER between the *Fusarium* spp. is not as sharp as one would infer from his paper. It is possible that these strains of *Fusarium* spp. are morphologically identical with those described by WOLLENWEBER, but physiologically unlike them. That this rule, if it exists, is not so rigid generally, however, is noted by SHERBAKOFF (**33**), who found that no correlation exists between morphological relationship and pathogenicity.

Although *F. oxysporum* is not absolutely unable to attack potato parenchyma, the potato tuber, in which usually only the xylem elements are invaded, enjoys an effective immunity from its attacks; and although *F. trichothecioides* can attack any subterranean part of the living potato plant, generally all parts excepting the mature tuber enjoy an effective immunity from its attacks.

The data given in the second part of this paper may furnish a partial explanation of these phenomena.

II. Ecology and physiology of the organisms

METHOD AND DATA

1. Temperature relations.—Observations of cultures grown at ordinary temperatures showed that there is a striking difference in the rate of growth of the two organisms. Potato cylinder, rice, liquid potato, and glucose media, and glucose and potato agar cultures all showed that *F. oxysporum* makes a considerably greater initial growth at temperatures above 20° C. than does *F. trichothecioides.* At temperatures in the vicinity of 10-15° C., however, *F. trichothecioides* makes the greater initial growth, although these temperatures lie below its optimum. The same difference was noted in cultures on neutral and acid potato agar. This point was also tested with cultures on sterile slabs of potato tubers kept in Petri dishes. At 25° C., *F. oxysporum* covered such slabs completely when *F. trichothecioides* barely had made a start, while at 12° C. the situation was reversed.

When 1 per cent liquid glucose media were inoculated with spore suspensions of *F. oxysporum*, visible growth was made in 16 hours; when *F. trichothecioides* was used, 30–42 hours elapsed before visible growth was made. This holds for temperature above 20° C. The optimum temperature for *F. oxysporum* was about 30° C., and for *F. trichothecioides* about 20-22° C., both varying slightly with the medium used. The maximum for *F. oxysporum* lay between 38 and 40° C. The optima and maxima were higher for cultures in potato extract than for glucose media cultures. The writer has not been able to determine the minima accurately because of inadequate apparatus. Humphrey (**15**) gives 4° C. as the minimum growth temperature for a certain strain of *F. oxysporum.*

Potato agar cultures of *F. oxysporum* and *F. trichothecioides* could endure a temperature of 40° C. for 5 and for 20 hours respectively and remain viable. Exposure to 50° C. for 5 hours killed *F. trichothecioides,* but not *F. oxysporum;* while exposure for 20 hours killed both. Some *F. oxysporum* cultures survived 5 hours exposure at 57° C.

The growth relations were also checked up quantitatively. In these experiments, as well as in all the following ones, the method suggested by Hasselbring (**14**) was followed. Erlenmeyer flasks of 200 cc. capacity were used with 50 cc. of solution per flask. The solutions in the flasks were autoclaved for 10 minutes at 7 lb. pressure, and then inoculated by means of sterile pipettes with a drop or two of spore suspension. The cultures were killed by adding 10 cc. of 10 per cent HCl to each flask. The cultures were then filtered off on tared Gooch crucibles prepared with asbestos, washed until acid free, and brought to constant weight in a Freas electric oven at 100° C., and the dry weight determined. It was found impossible at times to filter luxuriant cultures of *F. oxysporum* by this method, because of the tenacity with which this organism holds water. Consequently they were filtered on soft filter paper, transferred to tared Gooch crucibles, dried, and weighed The other organism holds water with little tenacity and filters with ease.

In all of experiments given below the following stock mineral solution was used: 20 gm. NH_4NO_3; 10 gm. KH_2PO_4; 5 gm. $MgSO_4$ per 1000 cc. H_2O. When carbohydrates were employed,

TABLE I

DRY WEIGHT (IN MILLIGRAMS) AFTER 20 DAYS' GROWTH IN POTATO EXTRACT MEDIUM; ROOM TEMPERATURE

	FUSARIUM OXYSPORUM					
	Temperature					
	35°	30°	25°	12°	1°.11*	−1°.11*
Flask 1	40	55	63	64	62	86
Flask 2	47	78	68	66		
Flask 3	61	86	80	68		
Average	49	73	70	66	62	86

	FUSARIUM TRICHOTHECIOIDE					
	Temperature					
	35°	30°	25°	12°	1°.11*	−1°.11*
Flask 1	0	0	60	87	146	83
Flask 2	0	0	64	100		
Flask 3	0	0	65	147		
Average	0	0	63	111	146	83

*For 20 days (no growth), then at 25° C. for 25 days.

these were added at the rate of 10 gm. per liter. Potato extract medium was made up by extracting 500 gm. ground potato tuber with 500 cc. H_2O, and then adding 500 cc. of the foregoing solution to the extract.

A series of cultures (table II) was run at 12° C. and the amount of dry weight formed determined at 2 day intervals for 10 days. In this series the medium was at 12° C. at the time of inoculation.

TABLE II

Dry weight (in milligrams) in potato extract medium; temperature 12° C.

	Fusarium oxysporum					Fusarium trichothecioides				
	Number of days					Number of days				
	2	4	6	8	10	2	4	6	8	10
I	0.2	0.4	5.2		13.2	1.4	4.0	9.4		27.0
II	0.6	0.4	5.6		13.0	1.8	4.4	12.2		44.6
Average	0.4	0.4	5.4		13.1	1.6	4.2	10.8		35.8

Table III shows the growth by day intervals made for 10 days when levulose was used as the carbon source. The solutions were at the temperatures indicated at the time of inoculation.

TABLE III

Dry weight (in milligrams) formed by day intervals

	Fusarium trichothecioides at 25° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I	0.2	0.6	4.2	12.8	29.0	19.2	25.4	19.4	34.0	43.6
II	0.2	2.4	18.0	31.6	32.4	34.2	27.2	41.9	47.7	50.8
Average	0.2	1.5	11.1	22.2	30.7	26.7	26.3	30.6	40.8	47.2

	Fusarium oxysporum at 25° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I	0.4	1.8	9.2	16.0	23.6	19.6	24.0	24.1	30.9	37.0
II	0.8	3.2	10.8	29.2	33.4	21.2	27.5	26.0	34.8	38.8
Average	0.6	2.5	10.0	22.6	28.5	20.4	25.7	25.0	32.8	37.9

TABLE III—*Continued*

	Fusarium oxysporum at 30° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I............	1.0	3.6	15.4	17.8	37.6	45.8	55.8	66.0	78.2	62.2
II	8.9	5.2	17.6	19.0	40.2	77.8	62.6	69.6	78.6	66.6
Average.....	4.9	4.4	16.5	18.4	38.9	61.8	59.2	67.8	78.4	64.4

	Fusarium trichothecioides at 25° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average.......	4.4	19	67.4					262		

	Fusarium oxysporum at 30° C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average......	11.2	48	108.6					240.2		

These tables show a tendency of *F. trichothecioides* to make a greater initial growth at low temperatures. At higher temperatures, however, unless above the optimum of *F. trichothecioides, F. oxysporum*, even though it made the greater initial growth, was soon overtaken and passed by *F. trichothecioides*. This was especially marked when dextrose and levulose were used as carbon source. This may be the result of a faster though more superficial feeding of *F. oxysporum*, which makes it unable to use materials as thoroughly as the other organism. This phenomenon is hardly a case of more rapid intoxication on the part of *F. oxysporum*.

The results obtained with artificial media were verified by infection experiments conducted with potato tubers kept at various temperatures. Tubers of the Red Cobbler variety were used. These were inoculated on April 1, and examined on May 27 (table IV). See figs. 11 and 12.

It should be noted here that *F. trichothecioides* when inoculated into a tuber can grow at 30° C., while it cannot do so in artificial media; and that *F. oxysporum* can survive a temperature of 1° C. in artificial media, but not in the tuber.

TABLE IV

CONDITION OF TUBERS AT CLOSE OF EXPERIMENT

Temperature	Fusarium oxysporum	Fusarium trichothecioides
30° C.	All completely rotted; sprouts killed	Slight rot in some
25° C	All completely rotted; sprouts killed	All completely rotted; some sprouts killed
12° C	All with very slight rot	All completely rotted
1° C...	No rot	All with slight rot
1° C. for two weeks, then 25° C. for two weeks	Slight rot in one tuber	All completely rotted
−1° C..........	No rot	No rot
−1° C. for two weeks, then 25° C. for two weeks.	No rot	All completely rotted

Discussion.—These results may, in part at least, explain why *F. oxysporum*, even though it can attack parenchyma and rot tubers, usually is not found in rotted tubers, while *F. trichothecioides* is. The ability of the latter to make a faster initial growth at the temperatures which prevail in the soil about digging time and in well kept storage places is probably the determining factor in this phenomenon. The experiments with tubers showed that *F. trichothecioides* made a great increase in growth rate when transferred from a low to a higher temperature.

These temperature relations may also explain in part the fact that we usually find *F. oxysporum* producing wilt under field conditions, and lend support to the observations made by ORTON (**27**), who reports potato wilt induced by *Fusarium* spp. to be preeminently a warm climate disease. *F. trichothecioides* can produce wilt, but the temperature conditions in the soil are such as to favor *F. oxysporum*, the maximum temperature of the former being the optimum of the latter. HUMPHREY (**15**), working in Washington on the tomato wilt induced by *F. oxysporum*, came to the conclusion that temperature differences in various parts of the state were determining factors for the appearance and non-appearance and severity of the disease.

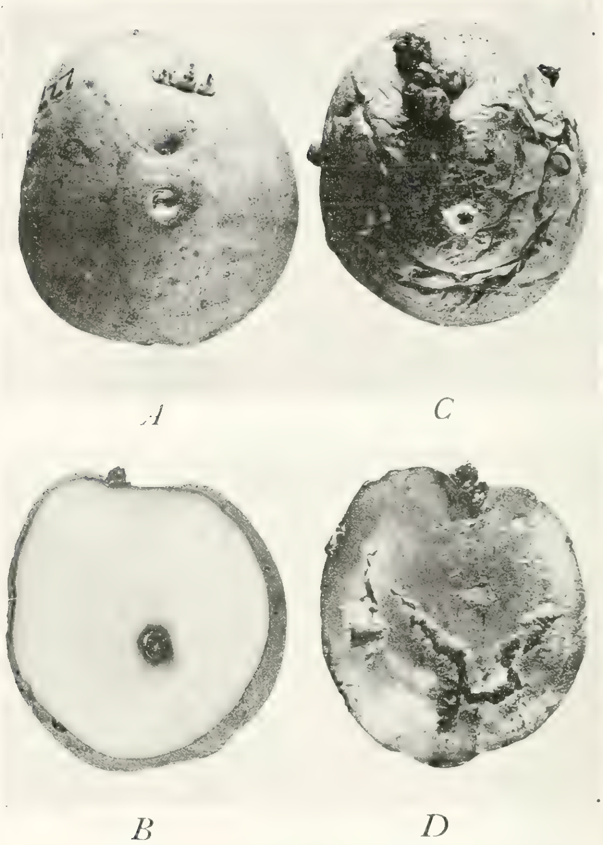

FIG. 11.—Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium oxysporum*, *A*, *B*, external and sectional view of same tuber, incubated for 30 days at 12° C.; *C*, *D*, external and sectional view of same tuber, incubated for 30 days at 25° C.

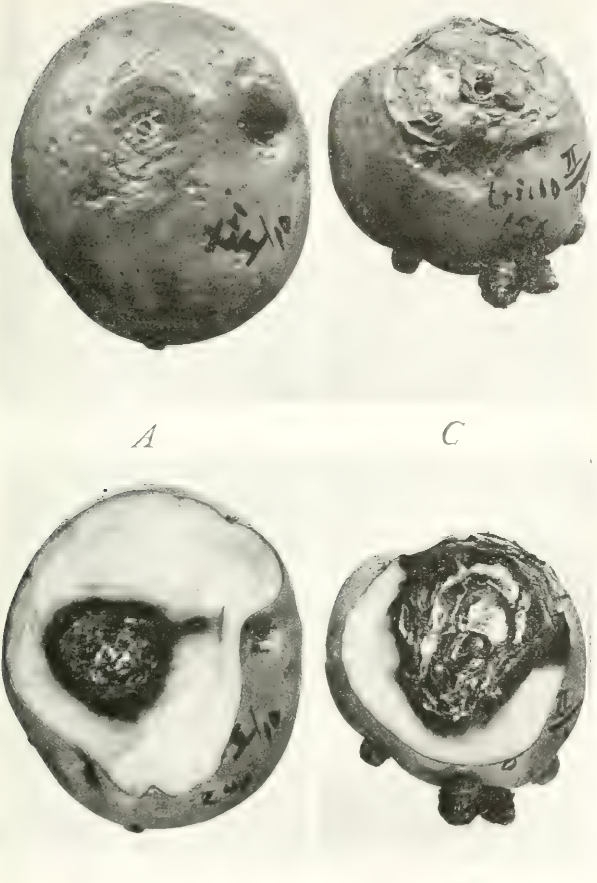

Fig. 12 – Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium trichothecioides; A, B,* external and sectional view of same tuber, incubated for 30 days at 25° C.; *C, D,* external and sectional view of same tuber, incubated for 30 days at 12° C.

2. GROWTH HABIT. It was observed in nearly all cultures that *F. oxysporum* not only made a greater initial growth at ordinary temperatures, but that it was at all temperatures much more of a surface grower than *F. trichothecioides*, making a superficial spreading growth, rather than the penetrating restricted intensive growth of the latter. Early sporulation was associated with the restricted growth habit of the latter (fig. 13). These habits were especially clearly marked on solid substrata, but even in liquid media *F. oxysporum* made a much less compact growth than the other species. It may be that the more spreading and extensive growth habit of *F. oxysporum* at all temperatures and its more rapid initial growth at temperatures above 10-15° C. are associated with a greater oxygen requirement than that possessed by *F. trichothecioides*. This would explain in part the frequenting of intercellular spaces and xylem elements by the former, and its consequent greater efficiency in causing vascular mycosis and wilt, as well as its tendency to cause bundle discoloration. The xylem elements of the stem end are undoubtedly infected while the tuber is yet in the soil, where temperature conditions are such as to favor the growth of *F. oxysporum*. Storage temperatures check the growth of this organism and the cells bordering the infected vascular elements shut the infected area off by suberizing their walls. Cultural experiments and microscopical studies show that cork is not absolutely impenetrable to these organisms, although it provides under normal conditions an effective barrier to the progress of both of these species. Because of the slower growth of *F. trichothecioides* at higher temperatures, the potato plant undoubtedly has a much better opportunity to guard itself by cork formation against this organism than against the other.

3. THE CARBON SOURCES OF THE TWO ORGANISMS.—A difference in the metabolic requirements of two organisms, a difference in their ability to utilize various substances, or a difference in their ability to tolerate the presence of substances may be factors of critical importance in determining which of the two will attack a given tissue or a given plant. These factors may determine also the modes of attack of an organism upon a tissue or a plant. Thus an organism that can digest pectinaceous material and not cellulose

would have to destroy a tissue whose walls are mainly cellulose by intercellular activity, while one that could digest cellulose might destroy this tissue by a primary cell invasion. Again, a greater ability on the part of an organism to digest suberin, other things

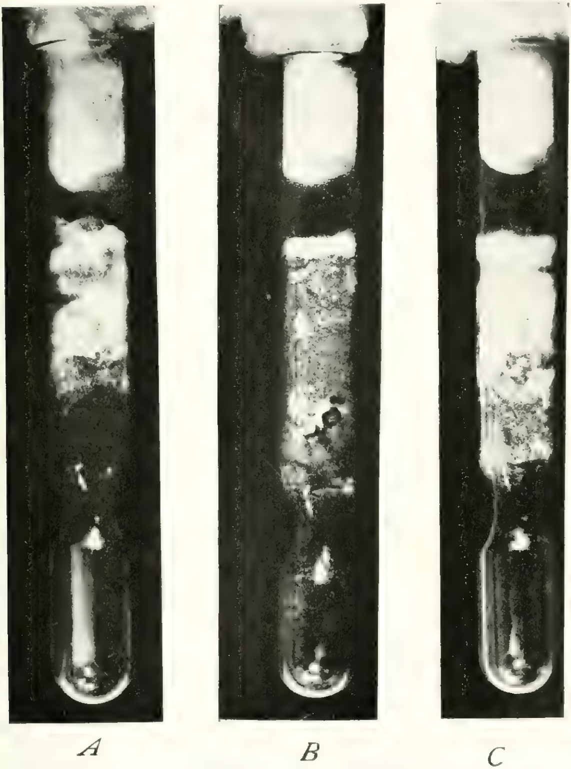

FIG. 13.—*Fusarium trichothecioides* and *F. oxysporum* on sterile potato cylinders; *A*, *C*, cylinders inoculated with *F. oxysporum*, incubated for 2 days at 25° C.; *B*, cylinder inoculated with *F. trichothecioides*, incubated for 2 days at 25° C.

being equal, would render it a much more formidable enemy of the potato plant than an organism without this ability, or possessing it to a less degree. This problem was attacked by making a study of the carbon sources of the organisms. The data reported here are only a beginning of this phase of the problem.

Fifty cc. of nutrient solution were measured quantitatively into 200 cc. Erlenmeyer flasks with a pipette. The flasks were then plugged with cotton, covered with tinfoil, and autoclaved. After cooling, 0.5 gm. of carbohydrate material was transferred quantitatively into each flask, and the flasks covered again with tinfoil and sterilized in a Freas oven by heating at 85° C. for one hour every 12 hours, for 6 consecutive days. The solutions were then incubated at 25° C. for 48 hours, so as to allow any contaminations present to appear. Low sterilization temperature was used to reduce hydrolysis of carbohydrates to a minimum.

The dry weight determinations were made by the methods outlined above. It was found advisable to kill two cultures of each set after 6 days, for the striking differences in rate of growth between the two organisms that were observed during the first 48-120 hours were obliterated by prolonged growth. The other 3 cultures were killed after 12 days' growth. The dry weight values do not show the differences in habit and rate of growth in the cultures as strikingly as they appeared to the eye. In many cases a visible growth was not determinable as dry weight. This is readily appreciated when we consider that moisture determinations indicated that the dry weight varied between 10 and 20 per cent of the wet weight.

In the controls, consisting of the plain mineral medium without carbon material, *F. oxysporum* made a weighable growth in 12 days, though not in 6 days, while *F. trichothecioides* made no weighable growth even after 12 days. Another important observation was made. In no case was it necessary to reinoculate with *F. oxysporum*, while many *F. trichothecioides* inoculations failed. The latter undoubtedly is the slower starter and much more poorly equipped for sure and quick infection than the former.

The figures in tables V-VIII represent milligrams of dry weight of material formed, except in those cases in which per cent is written. In such cases (cork, cellulose, and hemicellulose) the figures represent the percentage of decrease in dry weight of material. The last weighing in these cases unavoidably included the dry weight of fungus material formed, so that the figures are higher than they ought to be. The differences in weight in these cases give only comparative values of the amounts of material respired by the organisms. The filter paper used was the best Swedish paper, and the cork was obtained by skinning steamed potatoes, scrubbing the skin thoroughly, boiling it for 48 hours in distilled water, extracting for 48 hours in ether, and then boiling again with water. All figures represent averages, the composition of these figures being shown in tables VI and VII. In many cases there was a fair coincidence of the values, while in others a great disparity appeared. The averages probably would more nearly approximate the true value if a greater number of figures were available.

TABLE V

DRY WEIGHT IN MILLIGRAMS FORMED IN 6 AND 12 DAYS BY *Fusarium trichothecioides* AND *F. oxysporum*

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Ethyl alcohol		1.2	15.0	18.5
Glycerine	12.0	20.5	17.0	92.0
Mannit	108.5	136.3	109.5	112.0
Arabinose	5.2	35.0	78.7	43.0
Glucose	11.5	42.3	43.5	44.0
Mannose	2.0	52.0	49.0	62.0
Galactose	28.8	43.3	39.0	73.0
Fructose	56.5	81.0	74.5	77.6
Saccharose	9.5	45.6	36.5	35.6
Maltose	13.0	61.0	35.5	50.3
Lactose	1.3	20.0	4.0	21.0
Raffinose	12.4	47.3	44.5	54.6
Potato starch		45.0 per cent	81.0	103.0
Wheat starch	22.6 per cent	42.1 per cent	54.3 per cent	127.0
Corn starch	19.7 per cent	33.4 per cent	37.9 per cent	67.2 per cent
Soluble starch	45.2 per cent	49.0 per cent	110.5	210.3
Dextrine	4.4	37.3	42.0	56.3
Inulin	63.6	123.0	102.0	90.0
Gum arabic	1.5	18.6	37.0	58.3
Gum tragacanth	67.5 per cent	14.1 per cent	27.1 per cent	25.7
Hemicellulose	10.1 per cent	7.3 per cent	10.1 per cent	6.7 per cent
Cellulose	4.0 per cent	1.0 per cent	4.25per cent	6.7 per cent
Cork	10.1 per cent	5.9 per cent	12.8 per cent	3.9 per cent
No carbon source				0.5

Table V shows that qualitatively the two organisms behave alike in their ability to use all the carbon compounds tested. Quantitatively there is considerable difference, both as to rate of consumption and total growth after 12 days. *F. oxysporum* in general shows the greater speed of growth and greater growth after twelve days. In some cases *F. trichothecioides* shows the greater growth after 12 days.

TABLE VI

DRY WEIGHT (IN MILLIGRAMS) FORMED WHEN EACH FLASK RECEIVED 0.5 GM. OF MANNIT; CONCENTRATION 1 PER CENT

NO. OF FLASK	FUSARIUM OXYSPORUM		FUSARIUM TRICHOTHECIOIDES	
	6 days	12 days	6 days	12 days
I	93		107	
II	126		110	
III		108		130
IV		112		135
V		116		141
Average......	109.5	112	108.5	136.3

TABLE VII

HEMICELLULOSE (IN GM.) USED IN 12 DAYS

NO. OF FLASK	FUSARIUM OXYSPORUM			FUSARIUM TRICHOTHECIOIDES		
	6 days			6 days		
	Grams of material	Decrease in weight	Percentage of decrease	Grams of material	Decrease in weight	Percentage of decrease
I	0.5055	0.0455	9.0	0.5213	0.0513	9.8
II	0.5154	0.0584	11.3	0.4964	0.0524	10.5
Average			10.1			10.1
	12 days			12 days		
III.........	0.5185	0.0355	6.1	0.501	0.028	5.5
IV	0.5192	0.0382	7.3	0.5058	0.0488	9.4
Average			6.7			7.3

Table VIII gives the dry weight formed by the organisms when carbon acids were furnished as carbon sources. N/100 solutions were used, excepting for asparagin and asparagenic acid, whose solubility permitted only N/200 solutions. In the case of the higher fatty acids and oils, the material was weighed out as though N/100 solutions were being prepared. With these no weighings of the material formed were attempted, but merely differences in luxuriance of growth were recorded. To those acids which showed no growth with N/100 solutions, 5 cc. of 10 per cent glucose solution were added, making the sugar concentration 1 per cent, so as to determine whether the acid was merely non-usable, or whether it was toxic. Since it was found that some were toxic at N/100 concentration, lower concentrations were made up also. The results are given in table IX.

TABLE VIII

DRY WEIGHT (IN MILLIGRAMS) FORMED WITH THE FOLLOWING CARBON COMPOUNDS AS CARBON SOURCES

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Formic acid N/100	None	None	None	None
+5 cc. 10 per cent glucose solution.....	"	"	"	85.5
Acetic acid N/100...	"	"	1.2	10.6
+5 cc. 10 per cent glucose solution	"	106	None	None
Proprionic acid N/100	"	None	"	"
+5 cc. 10 per cent glucose solution	"	"	"	"
Butyric acid N/100	"	"	"	"
+5 cc. 10 per cent glucose solution	"	"	"	"
Glycocollic acid N/100.	2.5	7.3	20.0	8.6
Lactic acid N/100	0.3	0.6	8.0	23.0
Oxalic acid N/100	Non-weighable	Non-weighable	Non-weighable	Non-weighable
Succinic acid N/100...	Non-weighable	Non-weighable	8.5	9.3
Malic acid N/100	Non-weighable	Non-weighable	4.5	8.0
Tartaric acid N/100...	1.0	0.93	6.0	4.3
Citric acid N/100	2.6	6.6	8.0	6.6
Aspartic acid N/200	1.6		5.5	6.5
Asparagin N/200	3.5	3.8	5.5	5.3
Tannic acid 1 per cent	1.0	31.0	0.2	42.0
Tannic acid 0.5 per cent	1.6	61.0	1.6	41.0
+5 cc. 10 per cent glucose	3.5	32.0	5.0	47.0
Control 0.5 gm. levulose		82.0		116.0

TABLE VIII—*Continued*

	Fusarium trichothecioides 12 days		Fusarium oxysporum 12 days	
Palmitic acid I	No	growth	Good	growth
" " II	Slight	"	"	"
" " III	"	"	"	"
" " IV	"	"	"	"
+5 cc. 10 per cent dextrose, V	Excellent	"	"	"
Stearic acid, I	Slight	"	"	"
" " II	No	"	"	"
" " III	"	"	"	"
" " IV	"	"	"	"
+5 cc. 10 per cent dextrose, V	Excellent	"	"	"
Oleic acid, I	No	"	"	"
" " II	Poor	"	"	"
" " III	"	"	"	"
" " IV	"	"	"	"
+5 cc. 10 per cent dextrose, V	Slight	"	"	"
Palm oil, I	No	"	No	"
" " II	"	"	"	"
" " III	"	"	"	"
" " IV	"	"	"	"
+5 cc. 10 per cent dextrose, V	"	"	"	"
Olive oil, I	Fair	"	Fair	"
" " II	"	"	"	"
" " III	"	"	"	"
" " IV	"	"	"	"

A marked difference was found in the ability of the two organisms to use the fatty acids, *F. trichothecioides* being much more restricted in its ability. The experiments with alcohol and the acids also showed that the former organism was much more readily poisoned and inhibited in its growth. It was found that *F. oxysporum* grew well in 1 per cent ethyl alcohol, and that *F. trichothecioides* made no growth. The solution was then diluted one-half, whereupon *F. trichothecioides* made a good growth. This was clearly a case of inhibition. The growth of *F. trichothecioides* was inhibited by N/100 acetic acid, as can be seen by the fact that it grew in N/125 concentration and that it grew in N/100 when glucose was added, while *F. oxysporum* grew well in N/100 acetic acid. N/100 formic acid was toxic to *F. trichothecioides*, while it merely inhibited growth with the other organisms. The latter grew in N/125 formic acid, while *F. trichothecioides* did not grow in N/500 solution. N/100 proprionic acid was toxic to both, while both grew in N/250 solution. N/250 butyric acid was toxic to *F. trichothecioides,* while *F. oxysporum* grew in it.

TABLE IX

DRY WEIGHT (IN MILLIGRAMS) FORMED IN 12 DAYS IN VARIOUS CONCENTRATIONS OF ACID

CONCENTRATION	FUSARIUM OXYSPORUM				FUSARIUM TRICHOTHECIOIDES			
	N/1000	N/500	N/250	N/125	N/1000	N/500	N/250	N/125
Formic acid								
I	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	None	None	None
II	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	"	"	"
Average								
Acetic acid								
I					0.8	1.2	3.2	5.8
II					0.8	2	3.4	6.6
Average					0.8	1.6	3.3	6.2
Proprionic acid								
I	1.4	2.0	5.0	None	1.4	2.6	4.0	None
II	2.6	2.4	5.8	"	1.2	3.4	6.6	"
Average	2.0	2.2	5.4		1.3	3.0	5.3	
Butyric acid								
I	4.8	7.6	10.4	None	2.4	5.0	None	None
II	5.4	9.0	13.8	"	3.6	5.6	"	"
Average	5.1	8.3	12.1		3.0	5.3		
Oxalic acid								
I	0.4	0.4	0.4	1.2	0.8	Non-weighable	Non-weighable	Non-weighable
II	0.8	1.2	1.2	1.2	0.8	Non-weighable	Non-weighable	0.2
Average	0.6	0.8	0.8	1.2	0.8			0.1

A set of experiments was run also in which solanin in various percentages was added to glucose media. Because of the high cost of solanin, only 1 cc. solution was used in each test. The results are given in table X.

These differences, that is, greater versatility in the use of carbon sources, greater resistance to inhibition, and intoxication, may well play an important role in determining the difference in behavior of these two organisms.

TABLE X

DRY WEIGHT (IN MILLIGRAMS) FORMED BY *Fusarium oxysporum* AND *F. trichothecioides* IN 6 DAYS

	Percentage solanin				
	1	2	0.25	0.226	0
Fusarium oxysporum	10.6	8.1	13.2	10	13.8
Fusarium trichothecioides	2.5	2.4	7.8		9.3

Discussion.—The versatility of these organisms in using various carbon sources in their metabolism is of great interest. This almost omnivorous ability to use carbon compounds, including the simplest fatty acid, the highly oxidized fatty acids, the long carbon chain acids, the alcohols, mono- and poly-hydric, glycerin and fats, the mono-, di-, and poly-saccharides, including the dextrines, starches, hemicelluloses, and true celluloses, assigns to them an important role in the carbon cycle, and at the same time must help render them the formidable and destructive enemies of the root crops that they are.

The methods suggested by APPEL (**2**), namely, rigid inspection of potato fields, immediate destruction of all plants that show the slightest symptoms, quarantining of non-certified seed stock, alone give promise of keeping these troubles in check. Disinfection of storage cellars and of potatoes when put into storage, together with storage at proper temperature, will help combat these diseases, especially the dry rot induced by *F. trichothecioides.*

Conclusions

1. *Fusarium tuberivorum* WILCOX and LINK is the same as *Fusarium trichothecioides* WOLL.

2. Both *Fusarium oxysporum* and *F. trichothecioides* can produce both tuber rot and wilt of the potato plant.

3. The wilt is induced by destruction of the root system and by clogging of the xylem elements in the stem, and is, in mild cases, marked by such symptoms as discoloration of leaves, curling and rolling of leaves, and production of aerial tubers.

4. Under field and storage conditions *Fusarium oxysporum* is more probably responsible for wilt than is *F. trichothecioides,* and the latter more responsible for tuber rotting.

5. The optimum and maximum temperatures of *Fusarium oxysporum* are higher than those of *F. trichothecioides. F. trichothecioides,* however, grows well at 8–10° C., while *F. oxysporum* does not. These facts may explain in part the fact that *F. oxysporum* produces more wilt than *F. trichothecioides,* and that the latter causes more tuber rot.

6. *Fusarium oxysporum* has a more rapid, superficial, and spreading habit of growth than has *F. trichothecioides.* This may be associated with a greater oxygen requirement for *F. oxysporum,* and may account for the frequenting of xylem elements by this fungus.

7. Both organisms possess a striking ability to use the most diverse carbon materials as carbon sources in their metabolism. *Fusarium oxysporum* has a greater range in its ability, and can utilize the materials more readily, although not so completely as does *F. trichothecioides.*

8. *Fusarium oxysporum* is less subject to inhibition in growth and intoxication than is *F. trichothecioides.*

9. Solanin is not toxic to either organism, although it seems to inhibit somewhat the growth of *Fusarium trichothecioides.*

The writer acknowledges his indebtedness to Dr. E. MEAD WILCOX and to Dr. WILLIAM CROCKER. They not only made this research possible, but they gave freely of advice and criticism, and lent encouragement by their interest in the progress of the investigation.

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(WITH PLATES XIV-XVIII)

MABEL LEWIS ROE

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(WITH PLATES XIV–XVIII)

Introduction

Splachnidium rugosum Grev. is a monotypic genus of more than ordinary interest because investigators have placed it in one group or another according as some particular feature seemed more worthy of emphasis. The earliest mention of this plant was by LINNAEUS (**9**) as *Ulva rugosa;* a few years later SUHR (**18**) described it as *Dumontia rugosa;* and in 1830 GREVILLE (**3**) founded the genus *Splachnidium*, retaining the specific name already given.

Most authors have placed it with the Fucaceae, but in 1892 MITCHELL and WHITTING (**10**) published an account which was incorporated in 1895 by MURRAY (**11**) in his book. This account includes the morphology of both vegetative and reproductive tissues. As a result of their investigations, these authors felt justified in establishing a new family, making the following statement (p. 9): "The sum of the characters of *Splachnidium* so expressly excludes it from any existing natural order that there is no other course open to us than to establish one for its reception under the name of Splachnidiaceae." KJELLMAN'S account (**6**) restored it to the Fucaceae; and in 1904 OLTMANNS (**13**) placed it with the Ectocarpaceae under the subgroup Encoelieae.

The material for this investigation was secured by Professor CHARLES J. CHAMBERLAIN at Glen Cairn, near Cape Town, South Africa, on February 28, 1912. A second collection was made at the same place on August 30, 1914, by Miss EDITH STEPHENS of South African College, Cape Town, and sent to Professor CHAMBERLAIN. The work was undertaken with the hope of determining (1) the nature of the contents of the reproductive sacs, whether zoospores or gametes; and (2) the origin of the so-called "apical cell," a

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strikingly characteristic feature of *Splachnidium.* The material proved inadequate for the study of reproduction, but furnished a good series for the development and fate of the "apical cell." No mitosis was observed, and hence no chromosome count was possible. It is hoped that some one in the region where *Splachnidium* grows may be interested enough to investigate material secured at the exact time when cell division occurs, probably at night, or to observe the behavior of the living bodies after discharge from the reproductive sac.

Origin of the initial row

The first feature which tends to set the initial cells apart from adjacent border tissue is the taking on of a heavy coat of mucilage (fig. 1). In this and subsequent early stages the heavy mucilage coat is the only character which distinguishes the initial from young vegetative hairs abundant in the apical region (figs. 2–5). A second feature marking this unique structure is the beginning of resorption of cross-walls and loss of original definite nuclear structure (figs. 4, 6, 13, 15). Gradually a complete linear row of cells, reaching entirely through the thallus, is differentiated from the surrounding tissue as an initial row (figs. 5, 7, 8, 13, 15).

Sometimes there is no evident differentiation of tissue at an early stage, but portions of old apical hairs may assume the heavy mucilage coat (figs. 9–11), and there follows an involving of the entire linear row of which the hair is a terminal portion. Part of the old hair may be cast off, eventually leaving only the lower portion (fig. 11).

By the time an entire linear row is so differentiated, the characteristic filiform appendage, which has been described (**10**) as "growing between the cells of the thallus toward the center of the branch," begins to be evident (figs. 15, 16). Pressure of abutting tissue has narrowed the basal portion into a tail-like process as resorption of old walls and cell contents has gone on, whereas freedom from pressure above has allowed expansion into a pear-shaped oval. At this stage all of the original contents left in the filiform portion consist of granular patches of cytoplasm, and in the oval portion there is a similar granular substance with the addition of darkly staining bodies; there is no trace left of nuclear structure.

As active growth continues on each side of the initial row, rapid division occurs in the external layers of the thallus, whereas the inner cells, lying on the border of the central cavity, are pulled apart, not dividing to keep pace with the active outer tissue. This separation leaves the filiform basal process swinging loose, as it were, in the central cavity (figs. 17-20). The entire structure lies at the base of a slight depression, the result of arrested growth of the initial row followed by activity of abutting tissue.

Such, then, is the history of the initial row. *It is the result of more or less disintegration of an entire linear row of the thallus*, with perhaps the addition of portions of a terminal hair. *The exact amount of tissue originally involved*, whether including a terminal hair in part or in entirety, *determines the length and size of the mature structure*. For a long time the writer was puzzled by the great variation in length displayed by these bodies, and the explanation was apparent only when their origin was completely worked out.

The structure described in the foregoing is found in connection with any activity of the plant, as growth at the main apex, or production of lateral branches, or inception of conceptacles.

The conceptacle

In the formation of the conceptacle, the cavity in which the initial row lies becomes deepened as more and more of the abutting border tissue is involved. *Rapid radial division of these border cells contributes new tissue to the conceptacle at the same rate and in the same way as ordinary thallus tissue is developed.* In the early stages of the conceptacle, except for the initial row, there is no essential difference between adjacent tissue of the thallus and that of the actual cavity of the conceptacle (fig. 19). Presently, however, the lining cells of the conceptacle become elongated, and subsequently, by transverse division at the basal end, develop into septate hairs (figs. 20, 21). This behavior is exactly as noted by the writer in a recent investigation of *Fucus* (14) as the stage in the history of the conceptacle known as the "hair pit."

Some of the hairs, as in *Fucus*, are shed later and the lining cells put forth new papillate growths which elongate into sacs (fig. 22) which are ultimately filled with spherical bodies (fig. 24), variously described as zoospores (**10, 11**) and gametes. AGARDH (**1**), HARVEY (**4**), HOOKER (**5**), and LAING (**8**) all describe *Splachnidium* as dioecious, although no one of these authors reports having actually observed antheridia. LAING speaks of oogonia "obscurely pedicelled," developed from the lining of the conceptacle, each oogonium giving rise "to a large number of oospheres, thus differing from all other Fucaceae that have hitherto been described. Each oosphere is very small, compared with the oospheres of other Fucaceae." In another paragraph he states that "as antheridia have never been observed it is just possible that these hairs [apical hairs] may be antheridial in function." The writer's feeling is that the reproductive sacs contain isogamous gametes. However that may be, the conceptacles, at the time the reproductive bodies are ready for discharge, still contain the "initial" as a conspicuous feature (fig. 23).

Discussion

MURRAY (**11, 12**) and also MITCHELL and WHITTING (**10**) speak of the "modified cell" of *Splachnidium* as homologous with the "initial" of *Fucus;* and yet these same authors make this "unique cell" one of the reasons for separating *Splachnidium* from other families. The similarity holds true only in so far as the conceptacle is concerned, where, as previously shown by the writer (**14**), the conceptacle in each genus is the result of arrested development and partial breaking down of a portion of the external layer of the thallus followed by great activity of abutting tissue. The initial row is in no way comparable to the true apical cell of *Fucus*, segments of which are the basis of all tissue of the thallus.

The fact that the initial row of Splachnidium occurs wherever there is great activity is additional evidence for the simple origin of the conceptacle. *Splachnidium* is a particularly favorable plant for study along this line, since the same structure accompanies ordinary vegetative growth as well as reproductive activity.

In establishing a new family MITCHELL and WHITTING (10) summarize their reasons in the following statement (p. 8):

> While, then, the characteristics of *Splachnidium rugosum* are such that it can be placed in no existing natural order of the Phaeophyceae, its nearest allies appear to be the Fucaceae on the one hand and the Laminariaceae on the other. In its vegetative structure, in the nature of its thallus, and in the existence of conceptacles, *Splachnidium* bears a resemblance to the Fucaceae; it differs from them, however, in its mode of growth, the former increasing by means of an apical meristem, while the growth of the latter is due to division of an apical cell. In the presence of an apical meristem *Splachnidium* approaches the Laminariaceae, but at the same time there is no plant in this order which has a cell corresponding to the remarkable cell found at the apex of the main axis and lateral branches of *Splachnidium*. In its reproduction it is allied to the Laminariaceae, and the production of sporangia within conceptacles might seem to point to a narrower limitation of the fertile sorus of plants of this order, recalling the difference between perithecium and apothecium in the fungi.

As to vegetative growth, the tissue of the thallus of *Splachnidium* bears an equal resemblance to the Fucaceae and the Laminariaceae, the only essential difference being in origin. *Splachnidium* and the Laminariaceae are products of a meristem, the former apical, the latter intercalary, according to the evidence of most authorities, and not apical as described by MITCHELL and WHITTING in the passage just quoted. The Fucaceae, too, are characterized by apical growth of the thallus, but all tissue is the actual result of segmentation of a definite apical cell. In vegetative growth, then, *Splachnidium* is intermediate between the two groups.

The presence of conceptacles certainly seems a definite connecting link with the Fucaceae; and yet some of the Laminariaceae show a tendency to approach this method of reproduction. SMITH and WHITTING (17) describe deep furrows in *Macrocystis* and *Postelsia*, which closely resemble conceptacles in form and origin. In this feature, too, *Splachnidium* is intermediate between the Fucaceae and the Laminariaceae.

MURRAY (11, 12) and MITCHELL and WHITTING (10) conclude that the reproductive sacs of *Splachnidium* are neither oogonia nor antheridia homologous with those of the Fucaceae, but are sporangia homologous with those of the Laminariaceae, because of the size of the organ, its unilocular nature, its single wall, the number of spores contained, the size of the spore, and the persistent empty case. On the basis of these features there is no reason why *Splachnidium* may not equally well be considered as having unilocular gametangia containing isogamous gametes. This would give to *Splachnidium* a primitive position among the Fucaceae, but would offer for the Phaeophyceae a link in the phylogenetic sequence comparable to the position occupied in the Chlorophyceae by such plants as *Ulothrix*.

Moreover, in the Fucaceae the oogonia and antheridia are unilocular, no permanent walls separating the gametes. Also DREW (**2**) has reported conjugation of isogamous gametes in *Laminaria digitata* and *L. saccharina*. "The resulting zygospore divides and gives rise to a chain of cells which may represent the 2*x* generation, and this in turn gives rise to the *Laminaria* plant, which represents the gametophyte, or *x* generation." His observations have as yet neither been verified nor disproved.

Recently SAUVAGEAU (**15, 16**) has concluded from his investigations that *Sacchorhiza* (and probably all Laminarias) presents a heterogamous sexuality with alternation of generations. The large plant is the sporophyte bearing uniform sporangia, and each sporangium develops like zoospores "which after transformation into embryospores become male gametophytes or female gametophytes of microscopic size and independent for life. The oosphere expelled from the female gametophyte, then fertilized, germinates at once and develops the plant of *Laminaria*."

In the contents of the reproductive sacs, therefore, as well as in form and origin of the conceptacles and in vegetative growth, *Splachnidium* is intermediate between the Fucaceae and the Laminariaceae. There seems to be no justification for establishing a separate family (**10**) with its main character "reproduction by spóres contained in sporangia which are borne within conceptacles."

Further investigation will doubtless show a much more intimate connection between the Laminariaceae and Fucaceae than is at present recognized. *Splachnidium* may be placed with either group, since it has features common to both. Perhaps the presence of definite and conspicuous conceptacles, even though scattered indefinitely over the entire thallus, places it more closely with the Fucaceae. There seems no good reason for placing *Splachnidium* (**13**) with the Ectocarpaceae.

Summary

1. The initial row of *Splachnidium* is similar in origin and development to the "initial" of the Fucaceae, except that in *Splachnidium* it involves an entire linear row of thallus tissue; it may or may not include a terminal hair; and it accompanies vegetative as well as reproductive activity.

2. It seems unwise to place *Splachnidium* under a separate family; rather is it preferable to retain it under the Fucaceae, regarding it as a primitive member of that group for the following reasons: (*a*) it closely resembles the Fucaceae in the structure of the thallus, but has an apical meristem in place of a segmenting apical cell with consequent dichotomy; (*b*) true conceptacles are present which in origin and development are of the same general type as those of the Fucaceae, but are scattered indefinitely over the entire plant body; (*c*) the reproductive sacs may prove to contain isogamous gametes.

Acknowledgment is due to Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN for their suggestions and criticisms.

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EXPLANATION OF PLATES XIV–XVIII

All the drawings were made with the aid of the Abbe camera and reduced one-half in reproduction. For figs. 1–21, original magnification was 1580; for fig. 22, 790; and for figs. 23, 24, 1050.

FIG. 1.—Modified superficial cell, showing heavy coat of mucilage which distinguishes initial row from other border cells.

FIG. 2.—Two-celled stage of initial row.

FIG. 3.—Three-celled stage of same.

FIG. 4.—Two stages in development of initial rows; a two-celled stage at right and an older stage at left in which some of the cross-walls have been resorbed.

FIG. 5.—A linear row of the thallus, showing mucilage coat characteristic of an initial row.

FIG. 6.—An initial row in which cross-walls have been almost completely resorbed; traces of old walls still distinguishable.

FIG. 7.—A cut showing how initial row is usually terminated by sister cells, one of which usually fails to mature but sometimes initiates a young hair, as in fig. 8.

FIG. 8.—An initial row together with a young hair.

FIG. 9.—Basal portion of a branch of an apical hair which has become an "initial."

FIG. 10.—Lateral branch and basal portion of apical hairs which have become "initials."

FIG. 11.—Basal portion of an old apical hair in which cross-walls have been resorbed; the upper portion (unshaded) will be shed later.

FIG. 12.—An ordinary vegetative hair characteristic of apical region.

FIG. 13.—A completely involved linear row of the thallus with outer portion showing resorption of cross-walls and loss of nuclear detail.

FIG. 14.—Two initial rows which are modified young hairs.

FIG. 15.—An initial row including a linear row of the thallus together with a terminal hair; entire structure shows more or less disintegration and loss of cross-walls.

FIG. 16.—A stage which begins to show characteristic "filiform appendage" at basal end of initial row; almost all the cross-walls have been resorbed and original cell contents have disintegrated into granular patches.

FIGS. 17, 18.—Showing how interior cells of thallus pull away and release the "filiform appendage" from pressure.

FIG. 19.—A young conceptacle with young septate hairs.

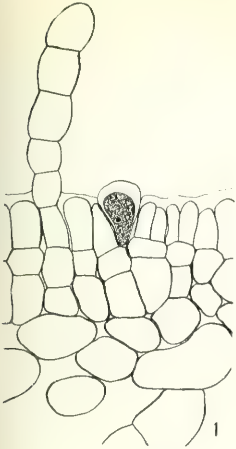
FIG. 20.—A later stage of a conceptacle with development of septate hairs.

FIG. 21.—The hair pit stage of the conceptacle.

FIG. 22.—A mature conceptacle, showing various stages of reproductive sacs (gametangia?) and a few fragile hairs; old hairs have been shed.

FIG. 23.—The initial row as it appears in a conceptacle at stage shown in fig. 22.

FIG. 24.—Gametangium (?) just before discharge of contents.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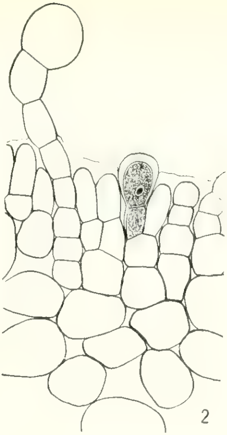
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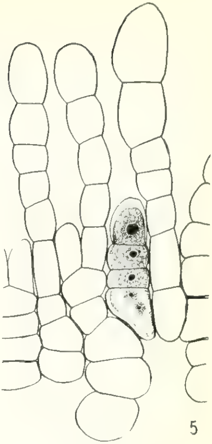
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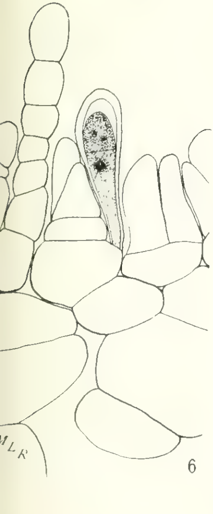

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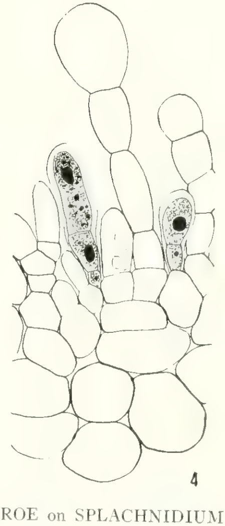
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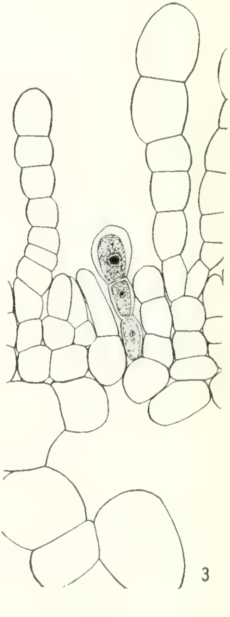
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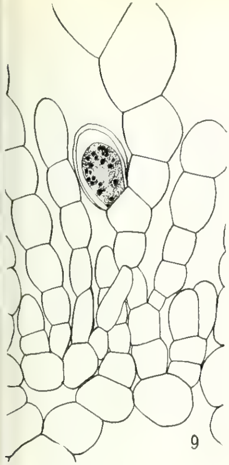
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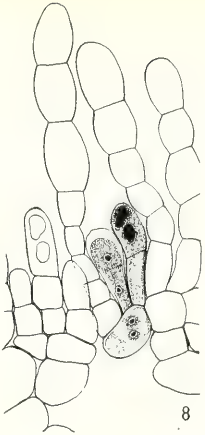
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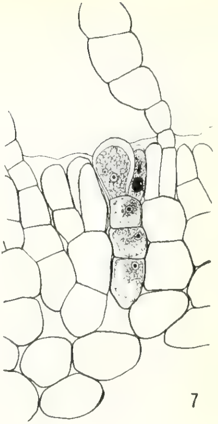
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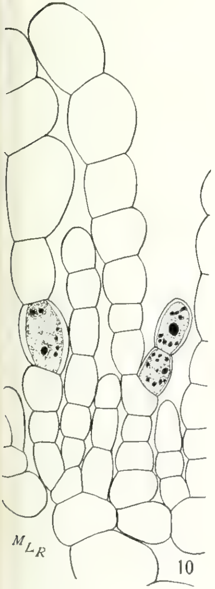

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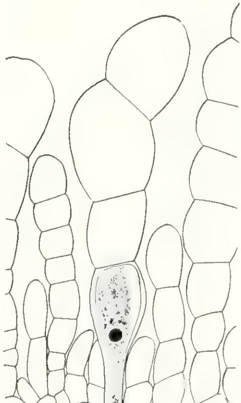
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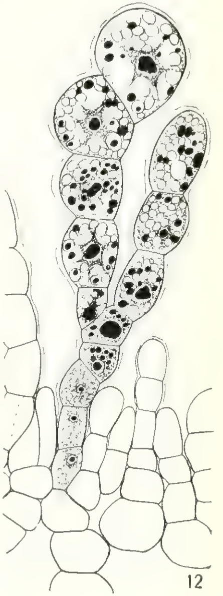
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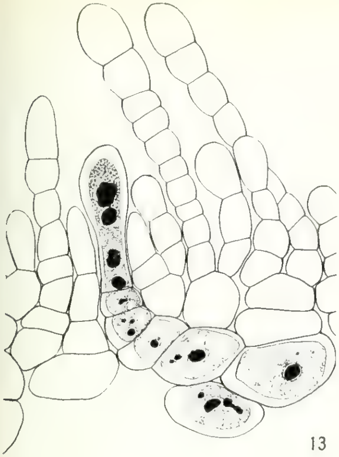

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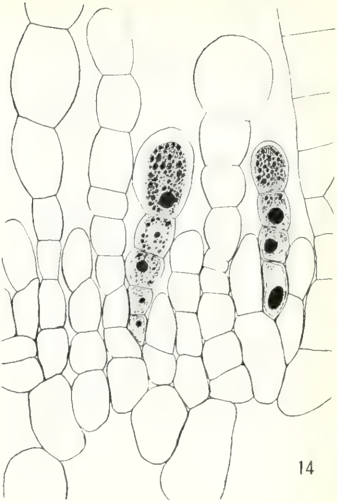

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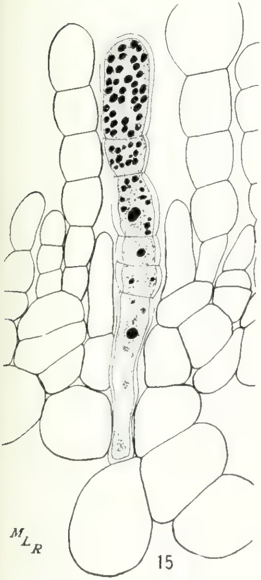

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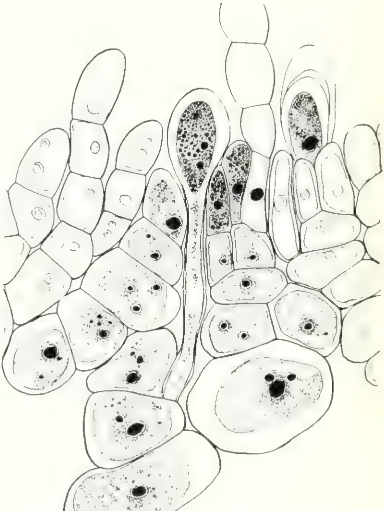

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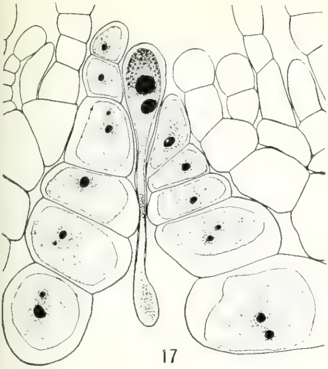

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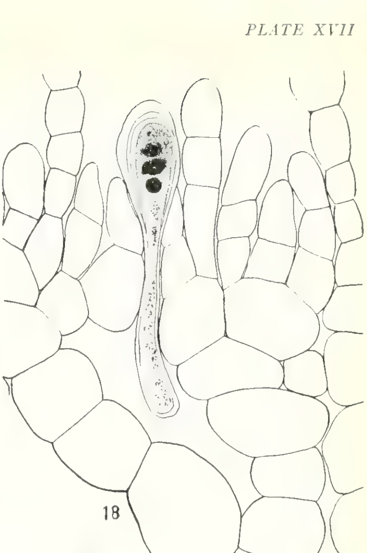

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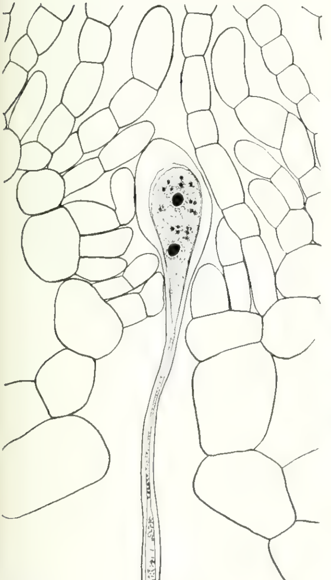

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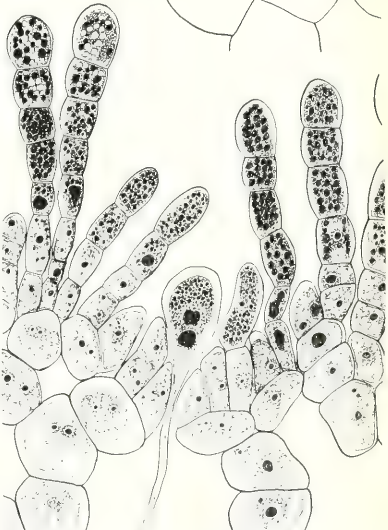

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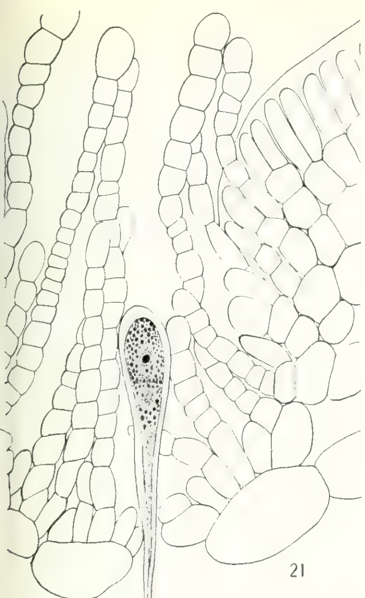

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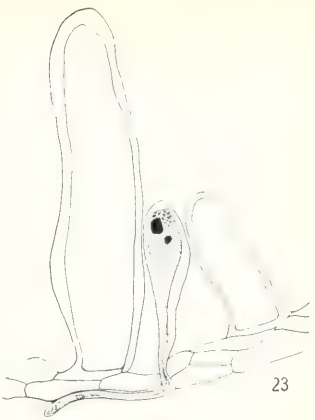

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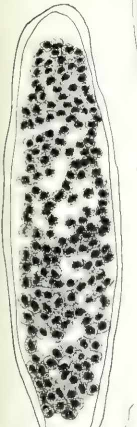

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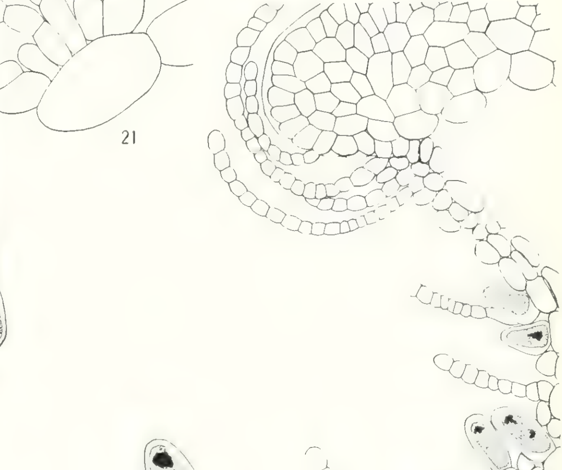

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ROE on SPLACHNIDIUM

THE EPIDERMAL CELLS OF ROOTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 221

(WITH SEVENTEEN FIGURES)

EDITH ADELAIDE ROBERTS

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Introduction

The epidermal cells of roots have received more or less attention, especially those epidermal cells which form root hairs. The lines along which investigations have been conducted may be grouped readily under investigations made when the root is in an air medium and those made when the root is in a liquid medium. The factors in the air medium which have been discussed are moisture, light, temperature, contact, length of cells, mode of succession, position of nucleus, osmotic pressure, membranes, and food supply. The factors in the liquid medium discussed are calcium nitrate, potassium nitrate, salt, and bog conditions.

MOISTURE.—PERSECKE (**16**), working with *Zea mais* and *Pisum sativum*, states that the root hair development depends upon the amount of air and water in the interstices of the soil. SCHWARZ (**20**), using the same forms, comes to the conclusion that there is a minimum of moisture at which the hair formation begins, an optimum in which the best development is obtained, and a maximum where the hair development nearly or entirely ceases. PFEFFER (**18**) and others attribute more importance to moisture than to light as a factor in hair development. The conclusion from these observations is that moisture is a factor in the determination of the formation or non-formation of an epidermal cell of a root into an outgrowth called a root hair, but how or why this is so receives no consideration, nor do the limits of the amount of moisture necessary to become a limiting factor.

LIGHT.—The effect of light upon cells in general was investigated by KRAUS (**14**), who found that darkness increased the length of cells. DEVAUX (**2**) found the same and that this favored

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the development of root hairs on corn. On the other hand, EWART (5), in the formation of root hairs on the root tendrils of *Vanilla*, found darkness accelerating and light retarding their formation. In this work, however, the moisture factor was not eliminated. PETHYBRIDGE (17) found that light retarded the formation of hairs upon oat and corn roots grown in water cultures. SCHWARZ (20) found that light and darkness had no effect on the root hair formation. SNOW (21) states that the effect of light and darkness, if any, is indirect. Light, then, as a factor has been experimented with to some extent with varying results, possibly owing to the fact that it has been associated with other factors.

TEMPERATURE.—No work on temperature as a single factor has been carried on. SCHWARZ (20) found that a temperature of 27–28°C. did not overcome the inhibitory effect of water as the roots grew smooth. SNOW (21), working with high temperatures plus moisture, found a decreased hair production brought about by the increasing elongation of the internal cells.

CONTACT.—The effect of contact has received some attention. SCHWARZ (20) observed that water roots upon entering the substratum develop hairs, and that when the soil is saturated the hairs on corn seedlings disappear, although soil particles are still present, and suggests that it may be due to chemical stimuli or retardation of growth, since it could not be due to contact. PFEFFER (18) denies that contact is a factor, for he found that on climbing roots hairs were produced on the side near the support where there is the greater moisture. SNOW (21) grew corn seedlings between plates and found no hairs on the sides touching the plates, while there were hairs on the other two sides; but again moisture may be the limiting factor and not contact.

JUEL (11) associated short cells and root hairs, as also did VAN TIEGHEM (25) and KRAEMER (13), and he finds that short cells remain short if they do not form root hairs. LEAVITT (15) finds two types of potential root hair cells. The first is that in which any cell of the outer layer may acquire the character of a root hair (trichome) by putting out a hair. This is characteristic of the dicotyledons, of some divisions of the monocotyledons, and of the Filicales. The second type is that in which they originate as specialized elements. These cells differ in the formation of their cell plate, the wall lies somewhat diagonally, and the cell differs from the other epidermal cells in shape, size, and content. This type he finds in the Schizaeaceae, *Equisetum*, *Azolla*, *Lycopodium*, *Phylloglossum*, *Isoetes*, *Selaginella*, and in Nymphaeaceae. SNOW (21) finds that no definite length can be given as the limit for the formation for hair development; that in some roots the average length of piliferous cells is less than that of the smooth cells, but that the differential elongation of the epidermal and cortical cells is important, and that hair formation depends upon their ratio, that is, between the capacity of the epidermal cells to elongate and their ability to do so.

BARDELL (1) comes to the same conclusion as SNOW. A few of the measurements given by BARDELL are shown in table I.

TABLE I

Plant	Length cortical cell (mm.)	Haired epidermal cell (mm.)	Hairless epidermal cell (mm.)
Pisum sativum......	231	161 (−71)	191 (−40)
" "	179	139 (−40)	172 (− 7)
Zea mais..........	69	66 (− 3)	77 (+ 8)
" "	69	69 =	77 (+ 8)
" "	40	55 (+15)	55 (+15)
" "	36	0	33 (3)
" "	124	58	62

It is difficult to draw the conclusion which is given, for in the one case a difference of 40 mm. in length between epidermal and cortical cells calls forth a root hair; while in another the same difference accounts for the absence of a root hair, and measurements equal to, or greater than, or less than, seem to account for haired or unhaired epidermal cells.

SUCCESSION.—SCHWARZ (20) found that hairs were always produced in acropetal succession, while DEVAUX (2) asserted that new hairs could appear among the old ones; whereas HABERLANDT (8) agrees with SCHWARZ (20), emphasizing the fact that no new hairs ever arise among existing ones.

POSITION OF THE NUCLEUS.—HABERLANDT (8) noted the position of the nucleus in *Brassica alba* to be at the tip. In *Pisum* *sativum* he finds that the protrusion of the root hair takes place opposite the nucleus. KÜSTER (12) takes exception to this. In the figures in SNOW'S (21) work the nucleus has no definite position.

OSMOTIC PRESSURE.—PFEFFER (18) found the osmotic pressure in corn root hairs to be greater than that of the cortical cells. STIEHR (23) found that root hairs on seedlings of *Spergula* which had grown in moist air when put in a 1 per cent magnesium solution burst at once, the nucleus being thrown out, and that always a greater percentage of the younger hairs burst. GANONG (7) found that the root hairs of *Salicornia* withstood 90 per cent salt water, *Suaeda maritima* 60–70 per cent, and *Atriplex patulum* 40 per cent. DRABBLE and LAKE (3) found that in mesophyll cells in plants growing in the same condition the osmotic pressure of the cell sap is generally the same, and in the plants of any area the osmotic pressure varies with the physiological scarcity of water. FITTING (6), using cells from the leaf, found that species showing high pressures in dry desert conditions show much lower pressures in moist situations, and concluded that certain plants adjust their osmotic pressure to the medium. ECKERSON (4) found that the root hairs were plasmolyzed by sucrose, varying from 0.20 μ to 0.30 μ. STANGE (22) found that in water cultures with nutrient solutions of high concentrations the osmotic pressures of roots are much higher than others; bean and pea in moist soil have pressures of 6.25N KNO_3 when growing in concentrated medium.

MEMBRANES.—SCHWARZ (20) found that the membrane of the root hair of *Taxus baccata* was of two parts: an inner layer which stains blue with chlorzinc iodide, and an outer layer which stains yellow brown. This outer layer is a mucilage which is hard in dry soil, and as moisture increases strongly swells and finally goes into solution. The mucilage layer gives no color with iodine and sulphuric acid, but stains red in an alcoholic solution of acid carmine. HESSE (9) measured the thickness of the membranes of the root hairs of many plants. He found that the thickness varied greatly with the family, but is nearly uniform within the family; that in any plant the thickness of any root hair membrane varies with the medium; and that in *Rosa canina* growing in a dry situation the root hair has a stiff pointed apex and that the membrane is of lignin, but that in moist air no lignin is found.

LIQUID MEDIUM.—*Calcium nitrate.*—SCHWARZ (**20**) found that 15 per cent of calcium nitrate inhibited hair production. KRAUS (**14**) found roots richly haired in calcium nitrate.

Potassium nitrate—BARDELL (**1**) found that the root hairs on *Zea mais, Triticum vulgare, Avena sativa*, and *Tradescantia* sp. tend to decrease as the fractional solutions of KNO_3 increase in strength from 0.01 normal to 0.09.

Salt.—HILL (**10**) found that in *Salicornia* and *Suaeda* the root hairs can regulate their osmotic pressure in proportion to the osmotic pressure of the soil water.

Bogs.—TRANSEAU (**24**), working on bog plants, found that *Larix* roots when not surrounded by water develop root hairs abundantly. RIGG (**19**) used the development of the root hairs of *Tradescantia* as an indicator of the presence of toxins.

FOOD SUPPLY.—SCHWARZ (**20**) found that in reducing the food supply by removing portions of the endosperm the length of zone of root hairs decreased. SNOW (**21**) experimented with *Helianthus* and found that those with the most cotyledon remaining had the best formation of root hairs.

Investigation

This investigation was prompted by the fact that the presence or absence of root hairs is so often used as an indicator of the effect of changed external conditions. That the effect of the varying conditions might be known, it seemed well to try to determine the varying factors within the epidermal cell of the root, as well as the effect of the varying factors without and their reciprocal relations.

In order to picture as definitely as possible all the forces which might be affecting the epidermal cell, we may consider the average epidermal cell as having the form similar to fig. 1. Here it is seen that on four sides (*2, 3, 4, 5*) the epidermal cell adjoins another epidermal cell, while on the inner side (*6*) it faces the wall of a cortical cell, and on side *1* it is exposed directly to the varying external conditions.

In fig. 2, which is a median section of fig. 1, the following factors need to be considered: (1) *air medium*, (*a*) the physical character of walls *1*, *2*, *4*, *6;* (*b*) the chemical nature of walls *1*, *2*, *4*, *6;* (*c*) the osmotic pressure on both sides of walls *2*, *4*, *6*, and inside *1;* (2) *liquid medium*, (*a*) the physical character of walls *1*, *2*, *4*, *6;* (*b*) the chemical nature of walls *1*, *2*, *4*, *6;* (*c*) the osmotic pressure on both sides of walls *1*, *2*, *4*, *6;* (3) *length of cells*.

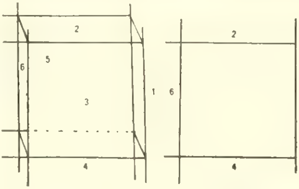

FIG. 1 FIG. 2

FIGS. 1, 2.—Fig. 1, form of an epidermal cell; fig. 2, median section of fig. 1.

TABLE II

Condition	Epidermal cell in mm.	Adjoining cortical cell in mm.
Haired	35	30
"	45	30
"	37	37
"	60	50
"	44	40
"	37	55
"	40	50
"	20	10
"	40	40
"	60	60
Unhaired	40	80
"	45–40	80
"	40	40
"	20	20
"	12	20
"	32	42

A few measurements were made of the length of walls of cortical cells in comparison with the length of adjoining haired and unhaired epidermal cells. These were taken upon the same corn root, the haired epidermal cells being those just above water and the unhaired just below water, with the results shown in table II. These measurements, although few, indicate that there is no definite relation between the length of the cortical cell and the epidermal cell which decides whether the epidermal cell shall become extended into an outgrowth or not. Fig. 3 shows a haired cell longer than the adjoining cortical cell, and fig. 4 shows two short epidermal cells adjoining one cortical cell. These measurements are corroborated by observations made throughout the work, many forms and all possible variation in length of cortical and epidermal cells being found.

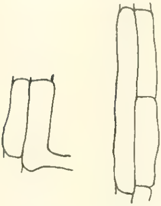

FIG. 3 FIG. 4

FIGS. 3, 4.—Fig. 3, haired cell longer than adjoining cortical cell; fig. 4, two short epidermal cells adjoining one cortical cell.

Position of nucleus.—The position of the nucleus has been found to have no relation whatever to the hair or to its initial formation. When the wall first swells, the entire wall bulges; later there is a greater swelling in one portion of it, the nucleus may not be near that portion; if the nucleus is near that portion, it may not follow the growth of the hair and be found near the tip of the hair, but remain in the base of the hair or in another part of the cell. There appears to be no relation between the position of the nucleus and the formation of the hair.

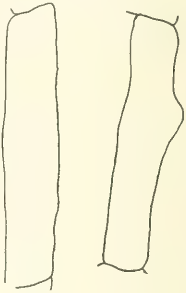

FIG. 5 FIG. 6

FIGS. 5, 6.—Fig. 5, bulging of entire wall; fig. 6, showing a narrowing of the swollen portion.

1. IN AIR MEDIUM

a) *The physical character of walls 1, 2, 4, 6.*—The first evidence of the formation of a root hair is the bulging of the entire wall *1*, as shown in fig. 5; then a portion of the wall stretches more than the rest and there is a narrowing of the swollen portion, as in fig. 6; but it may be noted that the base of a root hair is always greater in diameter than the rest of the hair, as in fig. 7. Seedlings of endive and lettuce demonstrate this fact. Branching of the hairs is common, indicating a difference in the physical character of the wall *1*. Sometimes the branch will be of less diameter than the main hair, as in fig. 8; and again branches will be of equal diameter, as shown in fig. 9.

The stretched condition of the wall is evidenced by the fact that in some root hairs which are grown in moist air the membranes burst when the hairs are placed in water, or in a solution which has an osmotic pressure lower than that of the root hair cell, as shown in fig. 10; whereas plasmolysis occurs when the root hairs are placed in a solution of higher osmotic strength (fig. 11). The root hair remains normal when placed in a solution of an osmotic pressure equal to that of the cell content, as in fig. 12.

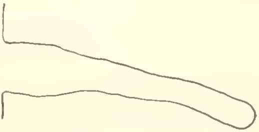

Fig. 7.—Base of the root hair greater in diameter than the rest of the hair.

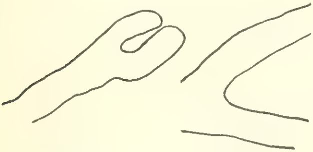

Fig. 8 Fig. 9

Figs. 8, 9.—Fig. 8, branch of the root hair of less diameter than the main hair; fig. 9, branches of equal diameter.

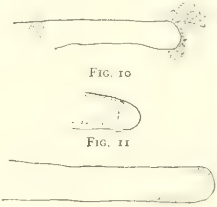

Fig. 10

Fig. 11

Fig. 12

Figs. 10–12.—Fig. 10, root hair grown in moist air and placed in water, membrane burst and content escaped; fig. 11, root hair which has been in a solution of an osmotic pressure higher than that of the root hair; fig. 12, root hair placed in a solution of an osmotic pressure equal to that of the cell content.

The following forms when grown in moist air and then mounted in distilled water were found to burst immediately: alfalfa, barley, cabbage, mustard, *Nasturtium*, radish, *Xanthium*, and wheat. These when mounted in a solution of sucrose of an osmotic pressure equal to the osmotic pressure of the cell in question remain unbroken, whereas plasmolysis occurs if a solution of higher osmotic pressure is used. The cells burst at the tip and a part of the content of the cell is ejected. The nucleus is thrown out if it happens to be at the tip of the root hair, but in case it is in or near the base of the cell it remains within the root hair. The break in the wall is immediately closed as the membrane springs back, and it is hard to detect the point of rupture except for the position of the escaped protoplasmic contents. The younger cells are more likely to burst than are the older ones. Fig. 13 (*a*) shows a radish root hair mounted in 0.24M sucrose solution; fig. 14 (*b*) is the same cell after being put in a 0.40M sucrose solution. It is to be noted that the walls corresponding to *2*, *4*, *6* are only very slightly changed, and wall *1* has decreased, thus showing that even in the same cell the physical condition of wall *1* is unlike that of walls *2*, *3*, *6*. In cells which do not burst when mounted in water, the surface of wall *1* is increased, as evidenced by a swollen tip which may or may not assume odd shapes. This indicates that wall *1* of these plants has a greater resistance, thereby indicating a variation in the epidermal cells of different plants, as well as a difference in wall *1* from walls *2*, *4*, *6*.

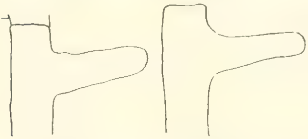

FIG 13 FIG. 14

FIGS. 13, 14.—Fig. 13, root hair mounted in 0.24M sucrose; fig. 14, root hair after being placed in 0.40M sucrose.

It is frequently seen in many forms, for example, alfalfa, cabbage, and *Verbascum*, that short root hairs are mingled with long ones, giving the appearance of younger and older ones being together (fig. 15). The difference may be one of time of formation or of a variability in the growth of wall *1*; at least in these forms the length of the hairs is not a grading one as seen in the forms usually figured.

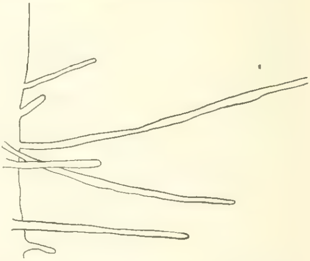

FIG. 15.—Short root hairs mingled with long ones.

b) *The chemical nature of walls 1, 2, 4, 6.*—It will be seen from table III that a majority of the forms investigated have a wall made up of two parts, an inner membrane which gives the cellulose reaction with 75 per cent H_2SO_4 and IKI, and an outer membrane which stains red with Ruthenium red, denoting pectin. That this membrane is calcium pectate is shown by the fact that on the addition of ammonium oxalate the membrane breaks down and calcium oxalate crystals are formed. It will be seen that some forms have a third membrane at the tip of the root hair. This membrane stains with all the callose stains, blue with aniline blue and resorsin blue, red with corallin soda. It will be noted that corn is the only form examined which has one membrane and that of cellulose.

TABLE III

PLANT	CHEMICAL NATURE OF ROOT HAIR MEMBRANES		
	Inner	Outer	At tip
Alfalfa	Thin cellulose	Thick pectin	
Amaranthus	“ “	“ “	
Barley	“ “	“ “	
Corn	Thick “	......	
Corn salad	Thin “	Thick “	Callose
Cabbage	“ “	Thin “	
Daucus carota	“ “	“ “	
Morning-glory	“ “	“ “	
Mustard	“ “	“ “	Callose
Nasturtium	Thick “	Thick “	
Pea	“ “	“ “	Callose
Sisymbrium	Thin “	Thin “	
Tobacco	“ “	Thick “	
Tradescantia	Thick “	Thin “	Callose
Vanilla	Thin “	“ “	
Ceraptopteris	Thick “	“ “	Callose

The walls *2*, *4*, *6*, and *1* are alike in that they all have an inner membrane of cellulose and an outer membrane of calcium pectate, but differ in that the calcium pectate membrane on wall *1* is thicker, and in some instances in the presence of callose at the tip of the hair. The calcium pectate membrane on side *1* is continuous with the middle lamella of calcium pectate of walls *2*, *4*, *6*. There is then a secretion of calcium pectate about the entire cell.

The relative thickness of the membranes having cellulose and pectin layers varies with the different plants, the cellulose layer being uniform over the hair, while the pectin is quite often thinner near the tip. Fig. 16 shows the tip of the root hair of *Nasturtium* with the two layers, the outer of pectin, and the inner of cellulose. Fig. 17 shows another *Nasturtium* root hair after it had been in ammonium oxalate; the calcium pectate has been changed to calcium oxalate and pectic acid. The calcium oxalate crystals are shown in the figure. The addition of Ruthenium red now gives no color except a faint tinge of pink about the crystals, due to the presence of pectic acid. Within the cell there are always masses of pectic substances; this is not calcium pectate, as it does not break down on the addition of ammonium oxalate, but still gives the color with Ruthenium red after treatment with ammonium oxalate.

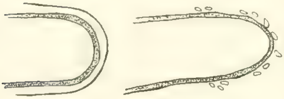

FIG. 16 FIG. 17

FIGS. 16, 17.—Two layers of the root hair membrane; fig. 17, *Nasturtium* root hair after being in ammonium oxalate.

Either of the membranes may be removed by treating the cell with their respective solvents. The calcium pectate membrane is soluble in 2 per cent HCl and 2 per cent KOH, the cellulose is soluble in copper-oxide-ammonia.

The fact that the soil particles are held to the hair by the transformation of the outer layer into mucilage has long been accepted, but what that mucilage is has not been known. In the case of corn growing in the soil or in quartz sand, the mucilage was found to be of cellulose, whereas in a *Coleus* root growing in the soil the soil particles are held to the hair by a pectin mucilage.

It has been known that the epidermal portion of the seedling from which hairs arise stains brown when put in a 2 per cent solution of potassium permanganate, and that the region just above that of the hairs is not colored by the solution. This has often been used to differentiate in a general way the stem and root regions of a seedling. The chemical nature of these walls was determined. The external wall of the epidermal cells above those forming root hairs has without the calcium pectate membrane on side *1* of the epidermal cell a third thin layer which is of cutin. It stains red with Sudan III and is insoluble in 50 per cent chromic acid. This wall *1* of the epidermal cell of the stem is then chemically different and physically of greater rigidity.

c) The osmotic pressure on both sides of walls 2, 4, 6, and on inside of wall 1.—The cortical cell is always plasmolyzed by a solution of sucrose which is 0.02M 0.04M stronger than that which plasmolyzes the epidermal cell; this indicates that on the outside of the wall *6* there is a greater pressure than on the inside; whereas on the outside of *2* and *4* there is a pressure equal to that on the inside. On the outside of wall *1* there is a pressure of one atmosphere. The variation between the outside and inside pressures of wall *6* is small, but there is a great variation in the pressures upon the two sides of wall *1*. Table IV gives the osmotic pressure of the root hairs of different plants grown under similar conditions.

TABLE IV

Plant	Osmotic pressure of root hair	Plant	Osmotic pressure of root hair
Alfalfa	0.24M sucrose	Lettuce	0.30M "
Amaranthus	0.30M "	Mustard	0.22M "
Barley	0.40M "	Nasturtium	0.28M "
Corn	0.28M "	Pea	0.22M "
Corn salad	0.28M "	Radish	0.28M "
Cabbage	0.28M "	Sisymbrium	0.24M "
Celery	0.30M "	Sunflower	0.22M "
Daucus carota	0.24M "	Turnip	0.30M "
Endive	0.24M "		

It will be noted in table IV that all are plasmolyzed by a solution of sucrose between 0.22M–0.30M sucrose, except barley. This is the equivalent of 5.10–7.70 atmospheres. Two facts are indicated here: first, that in moist air there is a minimum of difference of 4 atmospheres between the pressure upon the inside and upon the outside of wall *1*; and second, that under similar conditions there is a remarkably slight variation in the osmotic pressure of root hairs of seedlings of different genera. Some roots respond more readily than others to the percentages of moisture present in the media. Cabbage, alfalfa, squash, and corn show a definite response. The formation of the root hair is inhibited by removing the covers of the Petri dishes in which the seedlings are growing. Regions of no root hairs, corresponding to the growth made during the removal of the cover, followed in regions of hair formation at the period of its replacement.

A series of corn seedlings was grown in air of a known moisture content. This was controlled by percentage solutions of sulphuric acid. The corn was soaked for 24 hours and then hung on cork plates in bottles which were one-third filled with the varying solutions. The bottles, which were corked and paraffined, were all placed in a water bath kept at 24°C. The measurements in table V represent in each case the average of 5 sets.

TABLE V

Solution	Length of root in cm.	Diameter of root in mm.	Length of hair in mm.
Water	3.0	70	80
1 per cent H_2SO_4....	1.0	64	70
2 " " "	2.0	65	70
3 " " "	3.0	63	66
4 " " "	2.0	60	50
5 " " " ..	1.5	70	70
6 " " " ...	1.5	60	50
7 " " "	1.5	55	50
8 " " "	1.0	65	60
9 " " " ...	1.5	66	41
10 " " "	1.0	64	23
11 " " "	1.0	61	24
12 " " " ..	1.0	70	10
13 " " "	1.0	70	10

Table V shows that the cutting down of the moisture content affects the length of the root hair more than the length of the root or the diameter.

2. IN LIQUID MEDIUM

a) The physical character of the walls.—There is a variation in the wall of the corn in different media. In moist air the wall is uniform; in water when formed the hair is thickened at the tip.

b) The chemical nature of the walls.—The chemical study of the walls is under investigation.

c) *The osmotic pressure on both sides of 1, 2, 4, 6.*—The only wall on which the osmotic pressure may be made to vary at will is on the outside of wall *1*. Radish seedlings were grown in sucrose solutions of increasing concentrations in order to vary the osmotic pressure on the outer wall. The results obtained as the average of several series are given in table VI.

TABLE VI

Grown in	Diameter root in 1/100 mm.	Length hair in 1/100 mm.	Osmotic pressure	Difference between osmotic pressure of root hair and medium
Air	60		28	28
Water	40	50	38	38
0.02 molecular sucrose	40	30	42	40
0.04 " "	40	20	42	38
0.06 " "	40	40	42	36
0.08 " "	40	50	40	32
0.10 " "	20	20	40	30
0.12 " "	40	60	42	30
0.14 " "	55	60	44	30
0.16 " "	30	40	48	32
0.18 " "	0	10	0	0
0.20 " "	0	20	0	0
0.22 " "	20	10	50	38
0.24 " "	30	10	65	41
0.26 " "	30	20	60	34
0.28 " "	50	20	65	37
0.30 " "	40	40	65	35
0.32 " "	30	10	0	0
0.34 " "	40	40	70	36
0.36 " "	15	10	75	39
0.38 " "	20	10	65	27
0.40 " "	30	20	0	0
0.42 " "	20	15	0	0
0.44 " "	50	15	70	36
0.48 " "	70	15	75	29
0.48 " "	0	0	0	0
0.52 " "	50	20	80	28
0.60 " "	50	0	90	30
0.65 " "	50	10	100	35

It is seen that as the concentration of the media increases the osmotic pressure of the root hair increases almost in direct proportion. Thus the root hair maintains an osmotic pressure from 4 to 6 atmospheres above that of the medium.

Differential media.—The effect of different media on the two sides of a seedling root was determined. Petri dishes were filled with the solutions, seeds were attached to the edge, and the roots grew upon the solutions. The upper half of the root was in moist air and the lower half in the solutions. The roots grew straight, so that curvature could not account for the difference in the formation of the root hairs.

TABLE VII

PLANT	DIAMETER OF ROOT IN MM.	LENGTH OF HAIR (IN MM.)	
		In air	In solution
Helianthus	8	8	1 (0.25M sucrose)
"	8	5	1 (0.20M ")
"	5	5	1 (0.10M ")
"	5	5	1 (0.05M ")
Radish	5	8	2 (0.10M ")

Discussion

The measurement of the length of cell walls as an indication of differential growth seems of little value unless the number of the cells in any region is taken into account. Since there is not an epidermal cell corresponding to each individual cortical cell, it would seem that the sum total of the epidermal cells should be known in order to determine differential elongation as a factor. So far as known, the measurements taken were of individual adjoining cortical and epidermal cells.

BARDELL'S own measurements show that for corresponding epidermal and cortical cells there is no definite relation in length which would cause the epidermal cell to produce a root hair, as neither do the measurements found here on corn.

The factors which determine the initial swelling of the outer wall of the epidermal cell are of primary importance, while other factors come in which determine the later growth into a root hair.

It has been proved in root hairs that there are portions of wall *1* less resistant than others. The bursting of the root hair, the swelling, the branching, and the varying thickness of the membrane, all give evidence of this. The indications are that such weaker places exist in the membrane at the time of root hair formation, although this has not yet been proved definitely. These weaker regions would determine the region of the wall in which the localization of swelling takes place, which immediately follows the initial swelling of wall *1*.

The position of the nucleus can have nothing to do with the initial swelling, as the swelling is over the entire length of the wall *1*. Since the weaker places bear no relation to the position of the nucleus, the nuclear position can in no way affect the formation of the root hair.

In this discussion the second type given by LEAVITT is not considered, the type in which only specialized epidermal cells form root hairs. Preliminary observations indicate that the osmotic pressure of the short cells varies from that of the other cells, but there are doubtless other factors determining the hair formation.

Investigations so far indicate that there are two factors of importance in the initial formation of root hairs. One is the unequal pressure acting upon either side of wall *1*; the other is the variation in the physical character of the wall.

The difference in pressures on the two sides of walls *2*, *4*, *6* is so much less than the difference on the two sides of wall *1* that it is negligible. The osmotic pressure of the root hairs of the plants investigated when grown in moist air shows slight variation. In the plants examined the osmotic pressure approximates 5 atmospheres. The walls *2*, *4*, *6* have an opposing pressure equal to or greater than 5 atmospheres, but wall *1* must sustain a pressure of 4 atmospheres, for on this wall the internal pressure is opposed by only one atmosphere, when the root is grown in moist air. This pressure is sufficient to account for the initial swelling of wall *1*.

The result of increasing the osmotic pressure on the outside of wall *1* by growing radish seedlings in sucrose solutions of increasing osmotic value proved that the opposing pressures on either side of wall *1* still maintained a balance of at least 4 atmospheres in favor of an outward pressure.

Root hair formation is retarded when the moisture content of the air is decreased. This is shown by the experiments with seedlings grown in Petri dishes in alternating dry and moist air, and in those grown over sulphuric acid solutions. Reduced moisture affects the membranes by decreasing the extensibility of the walls due to an increase in the elasticity of the colloids. The osmotic pressure of the cell content is also increased by the modification of the moisture content of the air, but this is less effective because of the greater effect of the reduced moisture content upon the character of the wall.

The effect of reduced moisture content is again demonstrated in the experiments with differential media. In the case of *Helianthus* the effect of the sucrose molecule upon the colloids of the membrane and in the radish of glucose is to decrease the moisture content and the extensibility of the wall.

The wall is found to be composed of an outer membrane of calcium pectate and an inner membrane of cellulose. The only exception so far is in corn. A partial third membrane of callose is present in some. The amount of callose is so small and so little is known of its chemical nature that for the present it seems unimportant. The cellulose layer is important in that it gives greater strength to the wall.

The pectin layer and also the pectic substances within the cell are very important in the absorption and the retention of water, since pectin is a hydrophyllous colloid; and for this reason must influence the development of root hairs. The cations and the anions of the mineral substances in solution increase or decrease the absorptive capacity of this membrane according to a definite known manner, as indicated in the lyotropic series, investigations upon which should be made.

Summary

1. The initial formation of the root hair is indicated by a general swelling of the outer wall of the epidermal cell.
2. The swelling is produced if the physical resistance of the wall is overbalanced by the higher osmotic pressure which is maintained on the inside of the wall.
3. Further swelling followed by growth takes place at the less resistant portion of the wall.
4. This region bears no relation to the position of the nucleus.
5. The wall of the root hair is composed of two parts, an inner membrane of cellulose and an outer membrane of calcium pectate.

6. The presence of this membrane, together with the fact that the soil particles are held to it by a pectin mucilage, accounts for the high efficiency of the root hair as an absorbing organ.

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PROTHALLIA AND SPORELINGS OF THREE NEW ZEALAND SPECIES OF LYCOPODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 222

(WITH PLATES II AND III)

CHARLES J. CHAMBERLAIN

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(WITH PLATES II–III)

Few botanists have even seen the prothallia of *Lycopodium*, and most of those who have had such a privilege are indebted to one man, BRUCHMANN of Gotha; fewer still have made any investigation of the subject. The reason prothallia and sporelings of *Lycopodium* have not been so extensively studied as those of the ferns is not lack of interest, but difficulty in germinating the spores or finding prothallia growing naturally. In 1911 I collected adult plants of several New Zealand species of *Lycopodium* and made some effort to find prothallia, but my time was too limited for such slow, uncertain work. However, Professor A. P. W. THOMAS, at that time botanist of the University at Auckland, very kindly gave me prothallia and sporelings of 3 characteristic New Zealand species, *Lycopodium laterale*, *L. volubile*, and *L. scariosum*. Prothallia and sporelings are known in so few species that it seems worth while to give some account of this material.

Since the literature of the subject was examined with considerable care, all of the original papers cited being available, and since E. A. SPESSARD, a student of mine, is announcing in this issue of the BOTANICAL GAZETTE the first discovery of the prothallia and sporelings of several American species of *Lycopodium*, a historical résumé may be of service to those who are in favorable localities and who might wish to study the subject.

Historical

TREUB (7) introduced his classic account of the prothallium of *Lycopodium cernuum* with the remark that the history of the subject could be given in a few words, since it was necessary to cite only 3 or 4 investigators. SPRING (2), HOFMEISTER (3), DEBARY (4), FANKHOUSER (5), and BECK (6) are mentioned; but neither SPRING nor HOFMEISTER ever saw any prothallia. DEBARY, and later BECK, germinated the spores of *L. inundatum*, and FANKHOUSER found a few prothallia of *L. annotinum*.

TREUB overlooked the English surgeon, JOHN LINDSAY (1), who nearly 100 years before had germinated the spores of the very species with which TREUB was dealing. LINDSAY was also the first to raise ferns from the spores. Having noted very young ferns growing in the open in Jamaica, he sowed the spores ("farina") and watched their development. It was in connection with this work that he tried *L. cernuum*. His own account is interesting: "I have very lately sown that fine farina or dust contained in the anthers of a species of the genus *Bryum*, namely, *Bryum caespititium*, or one very like it, and also the farina of *Lycopodium cernuum*. There is a vegetable growth taking place where they were sown which I hope will prove to be their young plants." Later, in a letter to Sir JOSEPH BANKS in regard to *Bryum caespititium* and *Lycopodium cernuum*, he states that he had repeatedy sown them both, and in proper situation found that they grew very readily. There were no further figures or descriptions of either the *Bryum* or *Lycopodium*.

SPRING'S failure to germinate spores brought him to the curious conclusion that *Lycopodium*, and also *Psilotum* and *Tmesipteris*, consist exclusively of male plants, the females having been destroyed in some geological catastrophe. At this time it was generally believed that the spores, if they should germinate, would develop directly into the leafy plants. A few years later, HOFMEISTER opposed this view and predicted that the spores would give rise to prothallia bearing antheridia and archegonia, and that the leafy plant would arise from the fertilized egg; but all attempts to prove his theory by germinating the spores resulted in failure.

DEBARY succeeded in germinating the spores of *L. inundatum*, and in 9 days obtained prothallia consisting of 7 cells; but repeated attempts failed to produce more advanced stages, except that one prothallium was observed which had reached the 11-cell stage. The prothallia soon died.

In 1873, FANKHOUSER, in Switzerland, found prothallia and sporelings of *L. annotinum* growing naturally. This fortunate find, together with the results of DEBARY, who had described early stages of *L. inundatum* from cultures, made it possible to make a general outline of the development from the germination of the spore up to the adult prothallium with sporelings attached.

BECK sowed the spores of various species under various conditions, but *L. inundatum* was the only one to germinate and the prothallia did not get beyond the 10-cell stage. He asserted that after 2 years the spores of *L. clavatum* showed chlorophyll and looked as if they were about to germinate, but no cell division occurred.

Since FANKHOUSER'S paper appeared, 3 men have made large contributions to our knowledge of these peculiar prothallia. In 1884, 1886, 1888, and 1889, TREUB (**7, 9, 11, 12**) published a splendid series of researches upon the prothallia of Javanese species. In 1885, BRUCHMANN (**8**) began his patient and persistent researches upon the difficult temperate species with subterranean prothallia which had baffled all previous investigators; and in 1887, GOEBEL (**10**) found prothallia of *L. inundatum*, so that, with the stages secured by DEBARY, he was able to give a connected account of this species.

TREUB began his series with an investigation of *L. cernuum*. He germinated the spores of this familiar tropical species and some of the prothallia reached the early antheridium stage before they died. However, he found abundant material growing wild, and so had a complete series from the germination of the spore to the adult prothallium with embryos and older sporelings.

The prothallia are green and grow on the surface of the substratum, the largest reaching a height of 2 mm. When the spore germinates, a more or less spherical body is formed, about 8 or 10 cells in diameter. From the top of this body, which TREUB called the "primary tubercle," an alga-like filament then develops, at first consisting of a single row of cells, but soon dividing in all planes, so that a stout cylindrical body is formed more than twice the diameter of the primary tubercle and 5 or 6 times as long. The tip of the cylindrical portion is profusely branched, and at the base of the branches the antheridia and archegonia are borne. The embryogeny is particularly interesting, since the fertilized egg does not develop directly into a leafy plant, but produces a protocorm with protophylls, resembling a miniature *Phylloglossum*.

TREUB (9) next dealt with *L. Phlegmaria*, another familiar tropical species, epiphytic upon trees. The prothallia are found on the tree trunks just below the surface of the humus, but they have no chlorophyll, being entirely saprophytic and abundantly supplied with an endophytic fungus. The main body is tuberous, about 2 mm. in diameter, more or less spherical or somewhat elongated, and has several branches extending in various directions. The branches vary from 1 to 6 mm. in length and bear antheridia or archegonia or reproductive bodies which TREUB called "propagula." The antheridia and archegonia are usually at the tips of the branches, not at the base as in *L. cernuum*. The propagula are of two general types, one consisting of scores or even hundreds of cells forming a flask-shaped body with a slender stalk of one or two cells; the other is much smaller, more or less spherical in shape, and consists of only a few cells, usually not more than 2 or 3, with the outer walls very much thickened. In the first type the propagula break off at the stalk and grow directly into new prothallia, while in the second type there is a more or less prolonged resting period. The first type seems to correspond to the gemmae of liverworts and mosses, and the second type seems to correspond to the brown bulbils of mosses.

TREUB was not able to germinate the spores, and he believed that most of the prothallia found in nature come from propagula, prothallia from spores being comparatively rare.

The accounts of the development of antheridia, archegonia, and embryo are very complete, but the vascular anatomy of the sporeling is not described.

The prothallia of *L. salakense* are aerial, green, have no endophytic fungus, and are the only ones which TREUB succeeded in raising from the spore to the adult stage with antheridia and archegonia. In a few days the prothallia developed to the primary tubercle stage, then rested for several months, and finally resumed their growth and completed the life history. As in *L. cernuum*, a simple filament appears at the top of the primary tubercle and then forms a cylindrical body several cells in thickness, but other filaments then develop and behave in the same manner, so that there are several branches. The antheridia and archegonia are formed at the tops of these branches, there being no leaflike organs as in *L. cernuum* and *L. inundatum*.

The prothallia of *L. carinatum*, *L. Hippuris*, and *L. nummulariforme* are all of the *L. Phlegmaria* type, those of *L. carinatum* bearing such a close resemblance that TREUB (**11**) warns prospective collectors against collecting in localities where both species occur, since it is impossible to distinguish either the prothallia or the embryos. The prothallia of *L. Hippuris* are similar, but are more vigorous and the branches are thicker. TREUB was not able to disentangle complete prothallia of *L. nummulariforme* from the substratum and so had to write his account from fragments. He did not find any endophytic fungus.

The final paper in TREUB'S (**12**) series dealt with the embryo of *L. cernuum*. The series of stages was very complete, from embryos consisting of a few cells, through the protocorm stages, and up to sporelings with a few leaves. After the embryo has developed a protocorm with protophylls resembling a small *Phylloglossum*, a definite growing point is organized which develops into a leafy axis and at the same time the first root appears. TREUB indicated the course of the vascular bundles, but did not make any further study of the anatomy. He regarded the protocorm as a recapitulation of a *Phylloglossum* stage in the ancestry of *Lycopodium*.

In 1884, the same year that TREUB (**7**) began his research upon tropical forms, BRUCHMANN (**8**) found 3 prothallia of *L. annotinum*, and thus began a series of researches which extended over 25 years and resulted in clearing up the life-histories of the much more difficult temperate species. BRUCHMANN'S first paper appeared in 1885, but TREUB'S first account, although dated 1884, appeared at about the same time, so that neither knew the other was working upon prothallia. BRUCHMANN'S (**13**) most extensive work, which gained him the prize of the Paris Academy of Science, appeared in 1898, and contained descriptions of *L. clavatum*, *L. annotinum*, *L. complanatum*, and *L. Selago*. All were found growing in the Thuringer forest near Gotha, but the germination of the spores and earliest stages in the development were lacking. The development of antheridia, archegonia, and embryos are very clearly described. Ten years later this account was supplemented by a very complete description of *L. complanatum*. Although Bruchmann (**15**) had made repeated efforts to germinate the spores of various species, he met only the failures which had discouraged other botanists; but finally his perseverance was rewarded and he was able to give a complete account of the germination and early development of *L. clavatum*, *L. annotinum*, and *L. Selago*. The surprising feature is the long-delayed germination. The spores of *L. Selago* germinated in 3–5 years, and the development of antheridia and archegonia was complete in 6–8 years; *L. clavatum* and *L. annotinum* were even slower, germinating in 6–7 years and requiring 12–15 years to complete the development of antheridia and archegonia. Bruchmann suggests that possibly the periods might be shortened artificially if the proper stimuli could be discovered. All the species reported in Bruchmann's various papers are subterranean and saprophytic, but the spores germinate independently and develop to the 4 or 5-cell stage, and at this stage the fungus must enter or there will be no further development.

L. salakense, which Treub (**11**) succeeded in keeping in cultures throughout the whole life history, is green, aerial, has no fungus, and germination takes place in a few days. *L. cernuum* is also aerial and green and germinates with equal promptness, but it does not develop beyond the primary tubercle stages unless the fungus enters. *L. inundatum* in DeBary's cultures developed to an early primary tubercle stage with some chlorophyll and then died. The subsequent work of Goebel (**10**), who found prothallia growing naturally, proved that this species also has an aerial, green prothallium with an endophytic fungus. In *L. cernuum* and *L. inundatum*, however, the fungus infection is much slighter than in the saprophytic species.

So far as I have been able to determine, there is only one paper which makes any mention of the prothallia of New Zealand species of *Lycopodium*, and this paper by Holloway (**16**) deals primarily with the anatomy of the sporophyte. The investigation, both in the field and in the laboratory, is of such high grade that we hope Holloway will sometime give us an extended account of the prothallium and the anatomy of the sporeling. The varied species, ranging from epiphytes to ground forms, with prothallia ranging from the green, leafy aerial type to the deepest subterranean tuberous type, make New Zealand an ideal place for such a study.

Material and observations

The epiphytic type is not represented in the species at my disposal. *Lycopodium laterale* has a stout creeping rhizome, with numerous erect branches, and cones borne laterally; *L. scariosum* has a somewhat similar habit, except that the cones are terminal; *L. volubile* is the most beautiful species of the genus, bearing a striking resemblance to *Selaginella* as it trails along the ground or over bushes; but, unlike *Selaginella*, it keeps well after being gathered and is much used for table decoration.

PROTHALLIA

L. LATERALE.—The only reference I have been able to find in regard to the prothallium of this species is in HOLLOWAY's (**16**) paper. He says "in the case of *L. laterale* prothallial plants were found in two localities, growing on recently overturned marshy soil. The prothallus of this species corresponds to the type of *L. cernuum*, is small and short-lived, and is situated at the surface of the ground."

I had at my disposal 3 prothallia with protocorms attached and one older protocorm entirely free from the prothallium. In the first 3, each of the protocorms bore 2 fully grown protophylls; the older protocorm bore 10 protophylls. Two of the prothallia with their young plants are shown in figs. 1 and 2, the exact size being indicated in fig. 1*a*. The older protocorm with its 10 protophylls is shown in fig. 3. In fig. 1 the particles of sand and soil are not represented.

The upper half of the prothallium projects above the surface of the soil. There is a more or less spreading crown of leafy lobes, abundantly supplied with chlorophyll, and at the base of the inner face of these lobes the antheridia and archegonia are borne. It seems evident that the base of the prothallium was first to develop, but no sharply differentiated primary tubercle, like that shown in

TREUB's figures of *L. cernuum*, was found in these specimens. However, the base of the prothallium is more pointed than in *L. cernuum*, and this pointed base may represent the primary tubercle.

In proportion to the size of the prothallium, the protocorm is much more massive than in *L. cernuum*. There is no single, definitely organized growing point giving rise to all the protophylls, but rather a series of points, each giving rise to a protophyll. Stomata are abundant almost to the base of the protophyll; they are of the simplest type and open into a loose parenchyma with large air spaces. The transverse section is circular and shows a single weak vascular strand extending a short distance into the protocorm and ending blindly, without uniting with the strands of neighboring protophylls. The protuberance shown in front of the large protophyll in fig. 2 might be mistaken for the growing point from which the leafy axis is to be developed, but that point is formed much later, after several protophylls have appeared. The prothallium and protocorm shown in fig. 2 are similar, but indicate that there is considerable variation in both structures. Outlined against the protocorm is a second embryo.

The much older protocorm (fig. 3) indicates that the protophylls arise at irregular points, although there is a general progression, so that the protocorm resembles a very short horizontal rhizome. The 2 protophylls in the foreground are evidently the first ones formed, and the 3 much smaller ones at the left are the latest. The leafy axis of the permanent plant has not yet appeared. This specimen and also those shown in figs. 1 and 2 were sectioned, but the soil prevented satisfactory results. However, the sections showed the position of sex organs, the distribution of the fungus, and the relation of the protocorm to the prothallium. These features are shown, in a very diagrammatic way, in fig. 1*b*.

L. laterale belongs definitely to the type represented by *L. cernuum* and *L. inundatum*, since it has a short-lived green prothallium and an ephemeral protocorm with protophylls preceding the permanent leafy plant. *L. salakense* also belongs here, since the prothallium is green, and in its earlier stages behaves like that of *L. cernuum;* but it differs from the other 3 in having no endophytic fungus. Whether it has a protocorm stage is not known. TREUB failed to find sporelings when he made his first investigation; later, in his work on the embryogeny of *L. cernuum*, he figures a protocorm stage in *L. salakense.*

It is interesting to note that the protocorm stage has been found only in *L. cernuum*, *L. salakense*, *L. inundatum*, and *L. laterale*, all of which have spores which germinate, giving rise to green short-lived prothallia. The spore-bearing plants of all 3 species, as well as that of *L. salakense*, grow upon the ground. No green prothallium or a protocorm phase in the embryogeny has yet been reported for any epiphytic *Lycopodium.*

L. VOLUBILE.—The only reference to the prothallium of this species is by HOLLOWAY (**16**). He says "the prothallus is large, firm, and long-lived. Healthy prothalli were seen still attached to sporelings which were as much as 10 cm. in length. Generally the prothalli are subterranean, being buried 1–4 cm. in depth; in several instances, however, they were observed growing on the surface of the ground, and the upper portion of the prothallus was then well supplied with chlorophyll."

The material at my disposal included 9 prothallia and 2 sporelings, one of them still attached to the prothallium (figs. 4–9). All belong to the subterranean tuberous type, and 4 of them (figs. 4, 5, 8, and 9) show a primary tubercle. Although the material is somewhat limited, it is evident that there is considerable variation in size and form.

The endophytic fungus is most abundant midway between the center and the surface, and is entirely lacking in the crown, in the upper part of the depression within the crown, and in the axis of the prothallium. Cells with considerable fungus abut directly upon those with none at all, making a sharp contrast (fig 12). A detail is shown in fig. 13.

The crown is differentiated into two regions in some places, only the inner one of which bears archegonia and antheridia, as shown in figs. 5 and 6; but even in these 2 prothallia some portions of the crown show no such differentiation, and the prothallia shown in figs. 7 and 8 have uniformly rounded crowns with no indication of two regions. While most of the sex organs are on the swollen rim of the crown, they are not confined to this region, but occur in scattered patches within the rim on any part of the depressed region. A sectional view of a typical distribution of archegonia, antheridia, and the fungus region is shown in fig. 14.

The antheridia vary in size, shape, and output of sperms. They form hemispherical projections, with a nearly spherical mass of sperms; or they project scarcely at all, in which case the mass of sperms is not quite so regular. In a few cases, the sperm mass was elongated, making the topography bear some resemblance to that of an archegonium. In all cases, only one layer of cells separates the sperms from the surface, so that the essential course of development is uniform. A typical view is shown in fig. 15.

The foot of the sporophyte is strongly haustorial, and the cells surrounding it have some starch but very little protoplasm or other visible contents; consequently, the food supply must come largely from the fungus region and must be in a liquid condition even at a considerable distance from the foot. This is quite different from the condition in some gymnosperms, where only a single layer of cells may separate the haustorial cells of the embryo from those containing an abundance of food material in solid form. The foot is small and the vascular strand does not extend into it, but extends in an unbroken line from the shoot into the root, which is very late in developing. However, a few elongated cells, which do not become lignified, bend away from the main axis and point toward the foot.

L. SCARIOSUM.—The only description of this species is that given by HOLLOWAY (**16**), who says "the prothallus of *L. scariosum* was discovered in two localities. Like that of *L. volubile*, it appears to correspond to the *L. clavatum* type. It is large, firm, and long-lived, and in every case was found deeply buried (2–6 cm.)." Three specimens of this species were available and all had reached maturity, one bearing a young plant 18 mm. long, and the other two showing the foot and base of younger sporophytes which had broken off. Both prothallium and sporeling are larger and coarser than in *L. volubile*, as can be seen by comparing figs. 9 and 11, which are drawn to the same scale. The prothallium is densely infested by the fungus, which has about the same distribution as in *L. volubile*.

Origin of the subterranean habit.—That the green leafy prothallia represent the original type from which the subterranean forms have diverged can scarcely be doubted. The species with green, leafy prothallia (*L. cernuum*, *L. inundatum*, *L. salakense*, and *L. laterale*) have spores which, in the first 3 species, are known to germinate immediately; while in all those with subterranean prothallia the spores germinate only after a long resting period. It would seem that some change has occurred in the spore which has delayed the germination; and then only such spores as reached a protected situation would survive to germinate at all. Germinating in protected situations, with little or no light, the prothallia naturally would assume the forms of subterranean, dependent structures. That this has been the order of regression is indicated by the fact that the leafy crown has not been lost altogether, but only modified. In *L. annotinum*, as described by Bruchmann, the prothallium is subterranean and saprophytic, but still retains some of the leafy appearance; in *L. laterale* the crown is sometimes broken up into separate fleshy cushions which may represent leafy lobes; in more extreme cases, there is merely a swollen, fleshy rim to represent the leafy structure. The position of antheridia and archegonia is about the same as in the green, leafy forms.

If those who are expert in hastening the germination of seeds which normally have a long resting period, could find some way to make the spores of *L. annotinum*, or some such species, germinate immediately, it would not be surprising if green, leafy prothallia should appear.

ANATOMY OF THE SPOROPHYTE

In the vascular structure of the adult sporophyte *Lycopodium* still presents some difficult problems, although investigations like those of Hill and others have cleared up some of the phases. However, it seems likely that the final solution will come through a comparative study of sporelings, intermediate stages, and adult plants. Treub (7), Bruchmann (13), Miss Wigglesworth (14), and Holloway (16) have figured and described a few sections; but material has been scanty or other features of the problem have so engrossed the attention that this important feature has received little attention.

It would be dangerous to draw any serious conclusions from a study of 2 or 3 sporelings, all of which had reached the leafy stage; but, in the present condition of the subject, it seems worth while to describe a few features. The study was made from the sporelings shown in figs. 9–11.

In *L. volubile* the foot is quite small, and although somewhat larger in *L. scariosum*, no vascular strand extends into it, but a few cells, not lignified, point in its direction. The vascular strand extends in an unbroken line from the tip of the stem to the tip of the root, which in both species is late in appearing.

The sporeling of *L. volubile* shown in fig. 9, and that of *L. scariosum* shown in fig. 10, were sectioned transversely down to the crown of the prothallium, and the portion below the crown was then cut longitudinally. It would have been much better if transverse sections had been continued throughout. In both species the leaves are surprisingly like the protophylls of *L. laterale*. Throughout a considerable portion of their length the transverse section is circular, and even in the broader middle region the leaves are thick and spongy, consisting almost entirely of very loose parenchyma with large intercellular spaces and a single vascular strand. Stomata are irregularly scattered over the entire surface (fig. 16). The adult leaves in both species are rather thin.

In *L. scariosum* the shifting topography of the stele is a conspicuous feature, especially in the upper, leafy region; in the lower half of the sporeling, where there are only a few scale leaves with no leaf traces, the arrangement is more uniform. Near the middle of the leafy portion, a hexarch, pentarch, and tetrarch condition occurs within a vertical distance of 1 mm. Throughout the lower one-third of this specimen the stele is rather constantly tetrarch; but, just above the foot a few sections show a triarch and even a diarch stele. That the leaf traces connect with the protoxylem points is evident at a glance; but whether the leaf traces determine the topography is not so clear. However, it is significant that the stele is more complex in the leafy region and that it attains its greatest complexity in mature plants with larger leaves and vigorous leaf traces. In various places there are indications of the banded arrangement characteristic of the adult stele.

The differentiation of the vascular tissues is interesting. A short distance below the meristem the large cells which are to form the largest tracheids are easily recognized, and some of the cells of the points of the radial structure can be distinguished, although lignification has not yet begun. Very soon the points of the radial structure begin to lignify and are then marked off very sharply from the surrounding tissues (fig. 17). These patches of lignified tissue consist almost exclusively of coarsely pitted tracheids. It is possible that there are some spiral vessels, but it looks as if practically all of the spirally marked cells belong to the leaf traces. If protoxylem is to be identified by spiral and annular markings, very little of the tissue which becomes lignified at this early stage would satisfy such a criterion; but the tissue is so well defined and becomes lignified so far in advance of the rest of the xylem, and is so sharply marked off from the large cells which in longitudinal view have the typical scalariform marking, that it may very properly be called the protoxylem. It should be recalled that in the cycads spiral vessels in the protoxylem are largely confined to the seedling, the protoxylem of the adult plant consisting almost exclusively of tracheids.

The study of the root was not satisfactory. Near the tip the bundle is C-shaped and diarch with the phloem in the sinus.

The sporeling of *L. volubile* is comparatively slender and in every way more delicate than that of *L. scariosum*. In the upper leafy portion the stele is quite regularly radial and tetrarch; but from the secondary root (*r* in fig. 9) down to the prothallium the structure is regularly or irregularly triarch. In connection with the more uniform topography of this stele, it should be noted that the leaves and their single vascular strand are not nearly so robust as in *L. scariosum*. The adult stele has the banded arrangement. The differentiation of the tissues of the stele proceeds as described for *L. scariosum*.

Summary

1. *Lycopodium laterale* has a green, leafy prothallium, and there is a protocorm-protophyll stage in the embryogeny. *L. volubile* and *L. scariosum* have subterranean prothallia with no protocorm stage, but the early leaves have the structure of protophylls.

2. In *L. scariosum* and *L. volubile* the sporeling has a radial stele. The adult plants have a banded stele.

3. The outer part of the ray of the radial structure consists almost exclusively of pitted tracheids with scarcely any spiral vessels, but becomes lignified long in advance of the large tracheids of the metaxylem, and should be regarded as the protoxylem.

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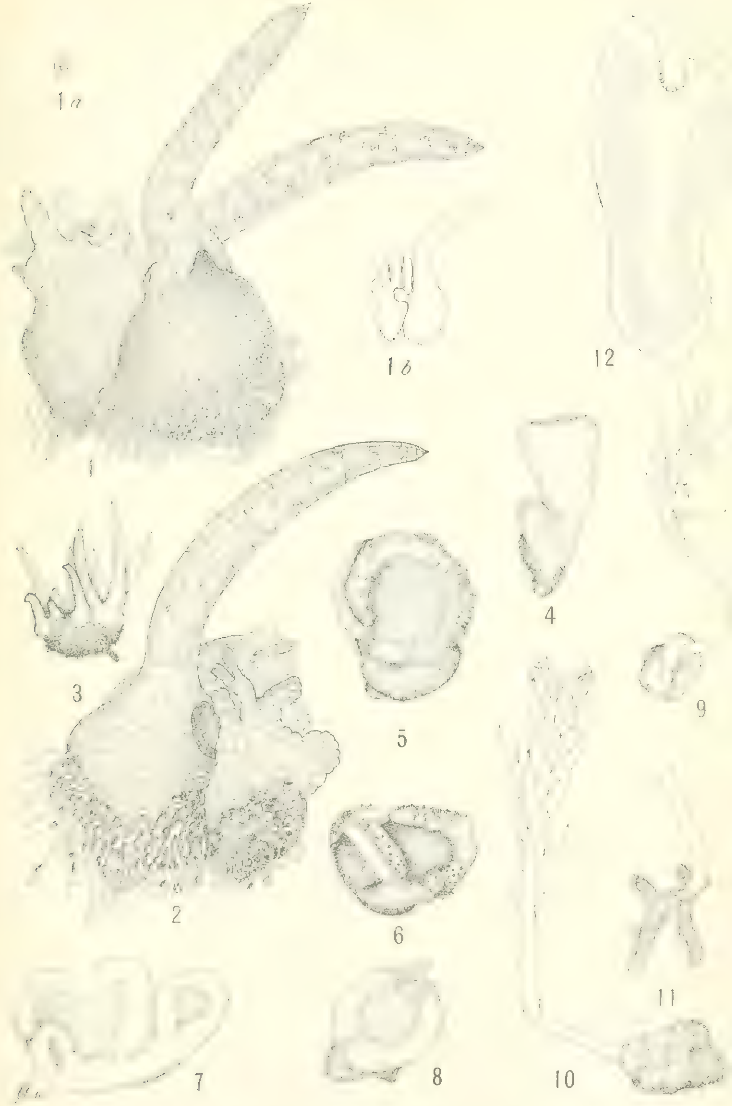
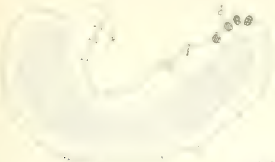


CHAMBERLAIN on LYCOPODIUM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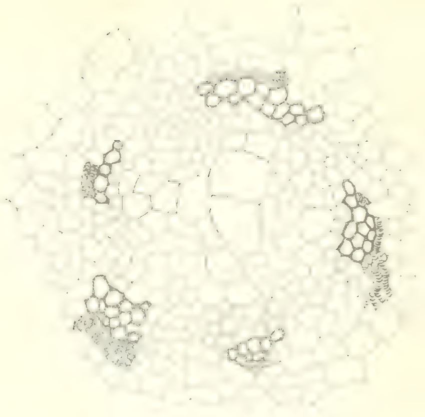
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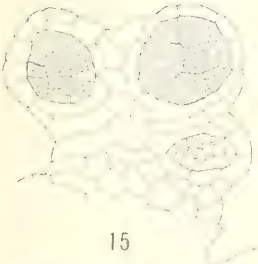
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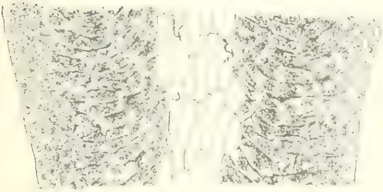
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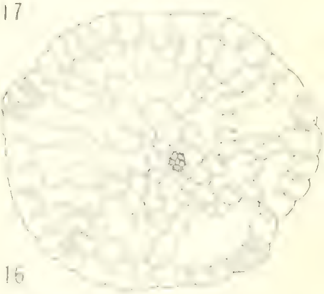
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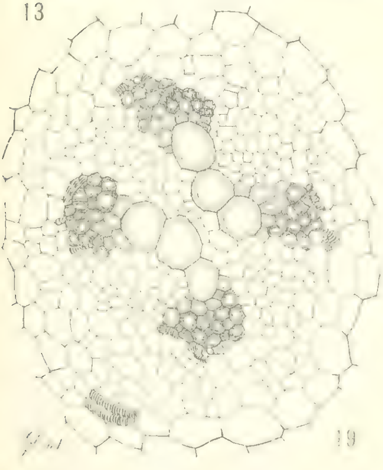
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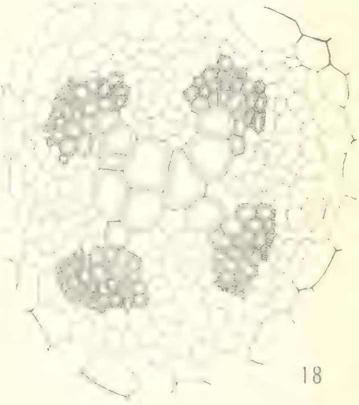
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EXPLANATION OF PLATES II–III

Fig. 1.—*Lycopodium laterale:* prothallium with leafy crown at left and bearing, at right, a protocorm with 2 fully grown protophylls and one young protophyll; ×20 (fig. 1*a* shows this plant natural size; fig. 1*b* is sectional view).

Fig. 2.—*L. laterale:* prothallium, at right, bearing a protocorm with 2 protophylls and also a second embryo; the soil and sand have not been removed; ×20.

Fig. 3.—*L. laterale:* protocorm with 10 protophylls; ×6.

Fig. 4.—*L. volubile:* prothallium showing primary tubercle at base; the crown is very even; ×10.

Fig. 5.—*L. volubile:* prothallium showing crown with numerous archegonia and antheridia; primary tubercle at base; ×10.

Fig. 6.—*L. volubile:* prothallium with archegonia and antheridia; the crown is lobed; ×10.

Fig. 7.—*L. volubile:* large, irregular prothallium; ×10.

Fig. 8.—*L. volubile:* prothallium with 2 young sporophytes; ×10.

Fig. 9.—*L. volubile:* prothallium with sporeling; a secondary root is shown at *r;* ×4.

Fig. 10.—*L. scariosum:* prothallium with sporeling; ×4.

Fig. 11.—*L. scariosum:* lower part of a young sporeling showing roots and foot; ×4.

Fig. 12.—*L. volubile:* diagrammatic view of prothallium with foot and lower part of sporeling.

Fig. 13.—*L. volubile:* detail of prothallium.

Fig. 14.—*L. volubile:* diagrammatic sectional view of prothallium showing archegonia, antheridia, and distribution of fungus, the latter indicated by shading.

Fig. 15.—*L. volubile:* portion of prothallium showing antheridia.

Fig. 16.—*L. volubile:* transverse section of leaf.

Fig. 17.—*L. scariosum:* transverse section of stem of sporeling before the large tracheids become lignified; *px*, protoxylem; *p*, phloem.

Fig. 18.—*L. scariosum:* same sporeling lower down; large tracheids have become lignified.

Fig. 19.—*L. scariosum:* same sporeling showing indication of banded arrangement.

PROTHALLIA OF LYCOPODIUM IN AMERICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 223

(WITH TWENTY-ONE FIGURES)

Compliments

Earle A Spe

EARLE AUGUSTUS SPESSARD

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EARLE AUGUSTUS SPESSARD

(WITH TWENTY-ONE FIGURES)

Since BRUCHMANN'S great work upon the prothallia of the European species of *Lycopodium* appeared in 1898, and probably before that date, numerous attempts have been made to find prothallia in America; but, so far as the writer has been able to determine, no successful searches have been reported.

While taking a correspondence course in botany with Professor CHARLES J. CHAMBERLAIN, he suggested that I avail myself of the opportunities afforded by my location in a *Lycopodium* region and make a thorough search for prothallia. In accordance with his suggestions and directions, the work was undertaken and has resulted in the finding of 21 prothallia and over 50 sporelings, representing 5 species: *L. clavatum*, *L. complanatum*, *L. annotinum*, *L. obscurum*, and *L. lucidulum*. It is also a pleasure to acknowledge my indebtedness to Dr. W. J. G. LAND for valuable suggestions.

The first specimen, that of *L. complanatum*, was found May 22, 1916. During the same month and in September of the same year, 6 more of this species, 8 of *L. clavatum*, 3 of *L. obscurum*, 2 of *L. annotinum*, and 1 of *L. lucidulum* were dug up, making 21 in all. Although the first sporeling bearing a foot, which indicated that a prothallium was recently present, was found on April 10, 1915, it was not until May 20, 1916, that a second was found 10 miles from it. During this interval approximately 150 days were spent crawling over the ground among and around the dense beds of adult sporophytes.

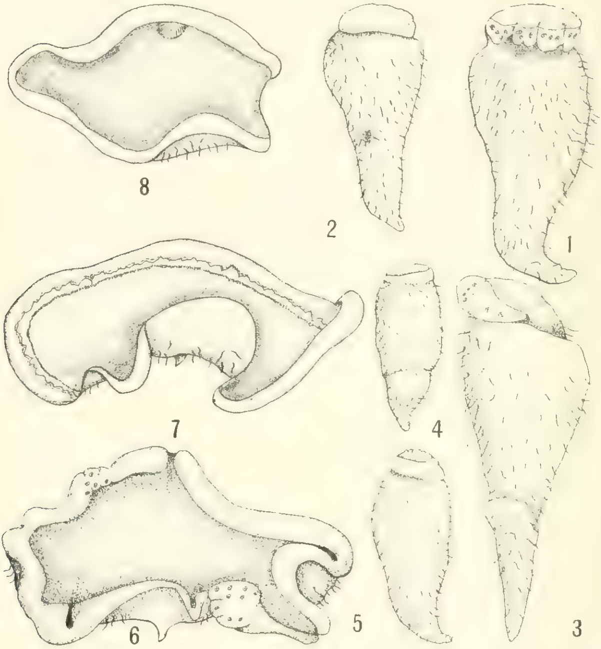
At first these tedious and futile efforts would seem to indicate that prothallia are rare. Yet, when it is noted that 17 of the 21 specimens were obtained from an area not more than 10 m. square, only about one-fourth of which was actually dug up; and furthermore, that as many as 6 were yielded by one small space 4 cm.

square, it seems probable that prothallia are rather abundant. The probability is even greater since the discoverer of these 21 prothallia was almost absolutely ignorant of the appearance of *Lycopodium* prothallia.

The discovery of the original locality was almost an accident. It is a place where one would least expect to find *Lycopodium* prothallia, at least those of *L. complanatum*, *L. clavatum*, and *L. annotinum*, since it is almost completely bare of adult sporophytes of these 3 species. I was wandering about looking for *Morchella*, when by chance I caught sight of an old sporeling of *L. clavatum*, growing in an exposed position between some wintergreen plants. An examination of the soil showed no prothallium. No adult sporophytes were within sight; but a sporeling is a good sign, and this encouragement led me to make the final successful search two days later.

Lying to the northwest of Marquette, Michigan, are a brewery and the remains of an old pesthouse. To the east of this pesthouse, about 700 m., is an open space bordering an open pasture on the north, a small wooded swamp on the east, and a wood composed of second growth poplar and maple on the west. A path runs to the northwest from the road which leads from Marquette to the pesthouse. Beside this path, at the distance indicated from the pesthouse, 18 of the 21 prothallia and most of the sporelings were found. The other prothallia and sporelings were found in two separate stations, the one half a mile, the other two miles from this one described.

The soil is mainly sand, covered in places by a very thin layer of humus; that within the edge of the wood is a black sandy loam. The specimens of *L. annotinum* and *L. lucidulum* shown in figs. 9 and 10 are the only ones which were found in the sandy loam where the ground is constantly shaded in summer. This specimen of *L. annotinum* is also enormously larger than the other prothallia, while that of *L. lucidulum* is decidedly the smallest. All the other specimens and most of the 50 sporelings were found in open, exposed, sandy places. The topography is uneven, rocks jutting up here and there between water-logged regions. Scattered about on the elevated regions are numerous small sandy knolls covered partly by *Polytrichum*, and sometimes by a species of grass. In many spots these knolls are absolutely bare save for a few plants of *Polytrichum*. It was in these knolls that all but 2 of the 21 prothallia were found, as well as most of the sporelings.



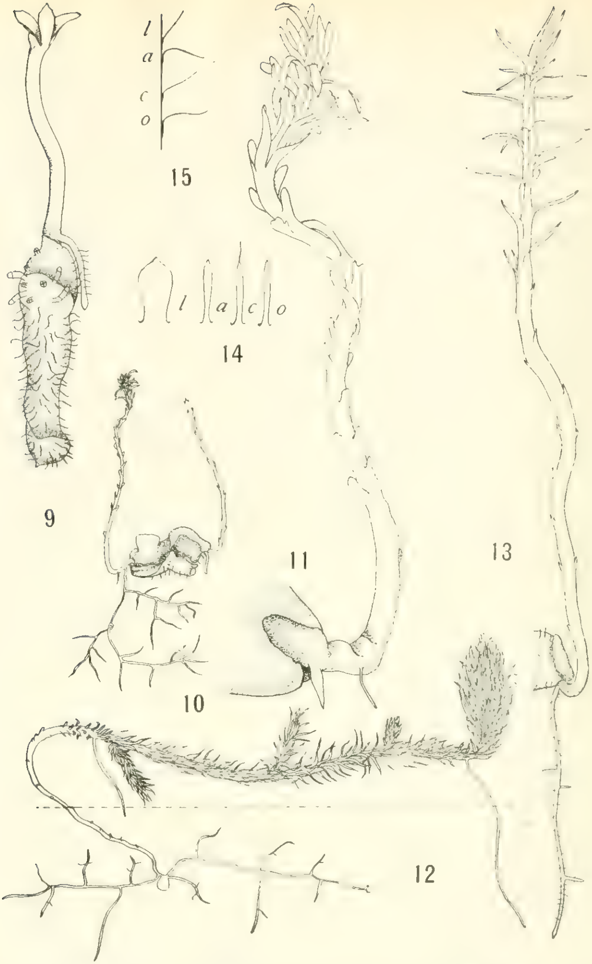
FIGS. 1–8.—Fig. 1, prothallium of *L. complanatum*, first specimen found, ×5; figs. 2, 3, prothallia of *L. complanatum* (?), showing openings to the antheridia which form the lobes entirely, ×5; figs. 4, 5, prothallia of *L. obscurum* (?), crown is double, ×5; figs. 6–8, prothallia of *L. clavatum;* in fig. 6 the antheridial lobes are marked as in figs. 1 and 2; in fig. 7 the lobed edge is double; ×10.

The frequent occurrence of sporelings and prothallia on these small, bare, exposed elevations suggests the idea that those spores which fall in such localities are first of all beaten into the ground by the frequent rains; later, they are covered over by the shifting surface sand, and are finally conveyed to a favorable depth by the percolating soil water. No experiments were made to see whether this is the correct interpretation; but it was this idea which led me to investigate the knolls. This observation was heeded in all searches subsequent to the first successful one, and all yielded one or more prothallia.

So far as the elevation of the knolls is concerned, too much importance should not be placed upon it. The fact that they are elevated somewhat and exposed as they are to the air currents merely causes them to be covered by leaves less frequently than the lower or more wooded regions near them, and consequently they offer a more receptive landing to the spores which are transported thither. This idea is corroborated by the fact that many sporelings of *L. lucidulum* and *L. annotinum*, as well as 3 prothallia, were found in the middle of a trail which is frequently used by hunters. In these instances the spores were clearly trampled into the soil until they found the depth required for their growth into prothallia. I suspect that future observations may show that the prothallia of *L. annotinum* and *L. lucidulum* require more moisture for their development than the other species mentioned. The 3 specimens I found of these 2 species, although the surface indications were similar, grew in decidedly wetter places.

In searching for prothallia, soil was removed with forceps. This method was not very satisfactory, however, and 4 of the prothallia were broken or pierced by the forceps before they were seen. It would be better to remove the soil to a depth of 10 cm. and wash it through a coffee sieve. This would not only avoid the danger of damaging prothallia, but would increase the probability of finding young stages. The specimens grew at depths varying between 1 and 7 cm. The species growing nearest the surface is *L. lucidulum;* the one growing deepest is *L. obscurum*. In one hole 2 kinds, *L. clavatum* and *L. obscurum*, were seen within 2 cm. of each other.

The prothallia of *L. clavatum* and *L. annotinum* grow with the wrinkled side toward the surface, and the primary tubercle pointed downward; that of *L. lucidulum* grows erect and the sporeling



FIGS. 9–15.—Fig. 9, prothallium bearing a sporeling of *L. lucidulum*, the only specimen found, showing 4 paraphyses, archegonia, primary tubercle, rhizoids, and an enlargement made by foot of sporeling, ×6; fig. 10, prothallium of *L. annotinum* bearing 3 sporelings, smallest one just emerging from upper tip of lower lobe of gametophyte, natural size; fig. 11, detailed drawing of largest sporeling shown in fig. 10, ×3; fig. 12, old sporeling of *L. clavatum* with foot still present, having lost erect habit of younger sporelings and assumed trailing habit of adult, ×2; fig. 13, sporeling and portion of gametophyte of *L. obscurum*, rest of gametophyte being like corresponding portions of fig. 5, ×4.25; fig. 14, diagram showing leaf contour of sporelings of *L. lucidulum* (*l*), *L. annotinum* (*a*), *L. clavatum* (*c*), and *L. obscurum* (*o*); fig. 15, diagram showing general profile of leaves of sporelings, the leaf being directed upward in *L. lucidulum* (*l*), drooping in *L. annotinum* (*a*), directed upward but bearing bristle in *L. clavatum* (*c*), bending downward then upward in *L. obscurum* (*o*), leaving the stem almost at a right angle.

arises straight out of the end lying immediately beneath the surface of the soil. The habit of growth cannot be stated definitely for *L. obscurum* and *L. complanatum*. The specimen of *L. complanatum* shown in fig. 3 was found in the same position as the specimen of *L. obscurum* shown in fig. 13. All the other specimens of *L. obscurum* and *L. complanatum* which I collected were disturbed before I saw them, and consequently their exact position was not determined, except the one in fig. 1 which grew vertically. In size, the prothallia of figs. 4 and 5, are very similar to that of fig. 13. This resemblance in size, together with a common external contour, are my only reasons for assuming that these 3 specimens belong to the same species. But here arises a difficulty. Although the prothallium in fig. 3 is about twice as large as the adult prothallium of *L. obscurum* (fig. 13) bearing a sporeling, we find the two growing in exactly the same position. This may mean two things; either the prothallia I have classed with *L. complanatum*, except no. 1, might as readily belong to *L. obscurum*, or the prothallia of *L. complanatum* grow in various positions in regard to the directions of their axes. If the axis of a prothallium of *L. complanatum* can be shown to grow always in a vertical position, and if that of *L. obscurum* can be shown to grow always in a horizontal position, the identification of the two species will then become a very simple matter if care be taken while hunting for them. I can only regret that this important point must be left for future observation to settle.[1]

Adult sporophytes of *L. clavatum* and *L. annotinum* were rare in this immediate locality, only one small patch of each having been found. A few plants of *L. complanatum* were found. *L. obscurum* and *L. lucidulum* grow in small clumps throughout the region.

The 50 sporelings belong mainly to *L. clavatum*, but a few were found of all the other species mentioned in this paper except *L. complanatum*. Since the sporeling is the first guide in the search for prothallia as well as in their identification, I have given 2 diagrams in figs. 14 and 15 to show the specific differences between the juvenile leaves of the 4 species of sporelings which I obtained. If the descriptions of these 4 species as given in GRAY'S *Manual* are followed carefully, the specific differences may readily be determined. The leaves of *L. clavatum* terminate in a bristle even in a stage as young as that sporeling of *L. annotinum* shown in figs. 10 and 11. I have not been able to separate sporelings of these 2 species at such a young stage or younger by any other character than by the presence or absence of the terminal bristle on the leaf. At such a stage the leaves have not yet assumed such very definite directions of growth as they do a little later, and as indicated in *a* and *c* of fig. 15. The sporeling of *L. obscurum* is easily identified by the 6 rows of leaves which early assume the characters peculiar to this species. Of course in the sporeling stage the leaves are fewer and more spreading than in the adult, so that a hurried examination would scarcely show the relationship between the two. I actually thought I was digging for a prothallium of *L. annotinum* while removing the soil from around the sporeling shown in fig. 13, and it was not until all the sand had been removed from among the rhizoids of the prothallium, and after the leaves had been examined under a lens, that I discovered my error. The sporeling of *L. lucidulum* cannot be confused in any way with the sporelings of any of the species I have mentioned. However, one may very readily believe that he is digging up a genuine one only to find that peculiar winged bud at the bottom of it. Yet, although I have experienced this disappointment a hundred or more times, through it I have observed that the sporelings bearing a foot grow just a trifle deeper in the ground. Generally the bud lies nearly or wholly on the surface, and unless it is rotted away, is readily seen. The sporeling of *L. lucidulum* looks exactly like one of the plants which grows from a bud, except that it has a distinct foot. Both bear leaves like an adult plant. Some of the plants in the vicinity are *Morchella*, *Polytrichum Acer*, *Populus*, *Pteris*, *Gaultheria*, *Rhus*, and *Polygala*.

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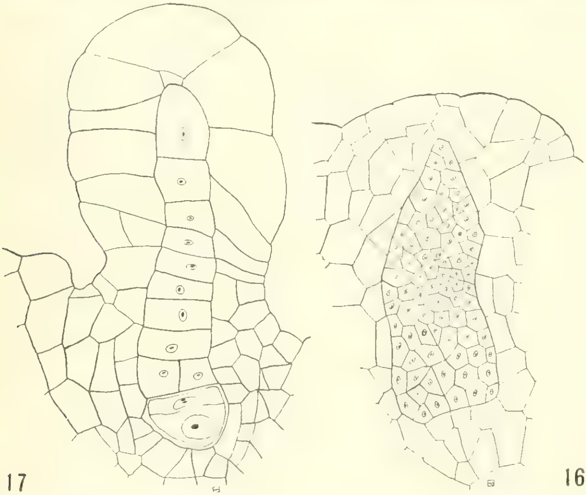
[1] On November 5, 1916, after this paper had gone to press, the writer found a large specimen of *L. obscurum* with sporeling attached. It was of the same color and shape as and grew horizontally like the prothallium labeled *L. complanatum* in fig. 3. This bears out the suggestion that all the prothallia classed with *L. complanatum*, except no. 1, very probably belong to *L. obscurum*.

The largest specimen of *L. annotinum* (fig. 10) measured 10 ×7 mm. It bore 3 sporophytes, one of which reached only 0.5

cm. above the ground, and another had just emerged from the prothallium. In color it resembled closely the gametophyte of *Botrychium virginianum*. The specimens of *L. clavatum* were much smaller and almost white in color. They varied in size from 6.5×5 mm. to 5×3 mm. The largest fragment measured 7×5 mm., and the smallest 2×1 mm. Figs. 6, 7, and 8 show the entire specimens. The fragments were more twisted and wrinkled than these, but both the fragments and the entire specimens showed a distinct crown on the border, which in one case (fig. 7) was double, the inner crown having an irregular outline. Each of the entire prothallia showed the primary tubercle very distinctly, near the middle, on the ventral side.



FIGS. 16, 17.—Fig. 16, antheridium of *L. clavatum* in sperm mother cell stage, ×365; fig. 17, archegonium of *L. complanatum* immediately before neck canal cells break down, the lowest neck canal cell being double, ×365.

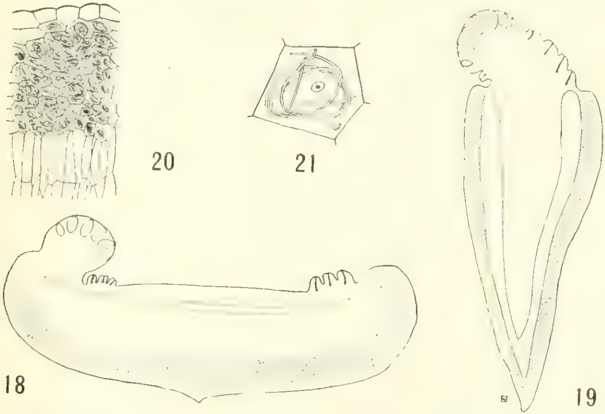
The prothallia of *L. complanatum* were all entire except one which had the lower portion broken off. Seven of these prothallia, illustrated by figs. 1, 2, and 3, bore a single crown which was more or less lobed. In section (fig. 19) these lobes proved to be masses of antheridia. The crown on each of the 3 prothallia of *L. obscurum* (figs. 4, 5) was double. An accident to the sections prevented a study of the relation between the lobes and the location of the sex organs in these prothallia. The crown was unequal in every specimen of *L. complanatum* and *L. obscurum*, and the lobes appeared only at one side, as shown in fig. 3.

The one prothallium of my collection which may cause some readers to question is that of *L. lucidulum*. I have found 10 sporelings and only one prothallium, but this single prothallium fortunately has a sporeling attached. This fact alone would not serve to convince a botanist in doubt of its genuineness. However, there are 5 reasons why I am convinced of its nature. Fig. 9 shows these 5 points. They are (1) archegonia, (2) paraphyses, (3) rhizoids, (4) a primary tubercle, and (5) the sporeling arises from it like the sporeling of any recognized gametophyte; there is a foot, and a primary root originates at the point where the stem breaks through. Certainly all these features cannot be connected with a young plant of *L. lucidulum* of asexual origin or with an associated fungus growth.

This prothallium of *L. lucidulum*, which, like that of *L. obscurum*, I believe is new to botanists, shows some very interesting evolutionary points. The body is somewhat cylindrical, but not entirely so. It is somewhat more flattened longitudinally than the figure shows. In this figure the flat side is turned toward the reader. Near the middle it makes a short twist toward the left. In general outline it looks like a prothallium of *L. complanatum* in the making, but on the upper lobed region are 4 paraphyses. Such a feature suggests a transition stage between the *Phlegmaria* type of prothallium, as represented by *L. Selago*, and such a form as *L. complanatum*. The specimen contained no chlorophyll so far as I was able to determine.

Among the 50 sporelings gathered there was a variation in age between 1 and 5 years. Each season's growth above the soil could be determined definitely by the alternate regions of longer and shorter leaves on the species *L. clavatum* and *L. annotinum*.

Fig. 11 shows in detail the largest sporeling of *L. annotinum* growing from the prothallium represented in fig. 10. The sporeling of *L. clavatum* shown in Fig. 12 was one of the largest, and serves to indicate the size one may obtain still bearing the foot. It is very possible, even for one who has studied carefully the figures of *Lycopodium* prothallia given in the various papers upon the subject, to mistake other forms of tubers for them. The sex organs may be indistinct or undeveloped, so that identification by means of them is impossible in such an instance. The number and variety of small tubers which grow in the soil of a wood are both large and confusing. Some of them are surprisingly similar in all outward appearances to small prothallia of *L. complanatum*. I do not doubt that I threw away some genuine specimens because they appeared to be tubers. I know that I retained some tubers and even sectioned them in paraffine because I thought they were prothallia.



FIGS. 18–21. Fig. 18, diagram of tissue regions in prothallium of *L. clavatum* (antheridia and archegonia indicated); fig. 19, diagram of median region of prothallium of *L. complanatum*, the fungus-infected region being indicated in this and fig. 18 by dotted shading; fig. 20, detailed sketch of fungus-infected region, showing its location beneath the epidermal tissue, ×75; fig. 21, single cell with endophytic fungus coiled within (fungus passes freely from one cell to another by piercing the cell wall), ×750.

To establish definitely whether the forms that I have collected are genuine prothallia of *Lycopodium*, therefore, I have drawn figs. 16, 17, 20, and 21 to show an antheridium, an archegonium in which the lowest of the neck canal cells is double, and 2 detailed sketches of the fungus-infected region. The diagrams in figs. 18 and 19 indicate the tissue regions shown by sections of 12 prothallia.

This article seeks to establish the fact that the prothallia of *Lycopodium* have been found in America; to make known to those botanists who may later desire to find prothallia for themselves, what the conditions were under which the specimens collected were found; and to announce the discovery of 2 new species of *Lycopodium* prothallia, namely, *L. obscurum* and *L. lucidulum*. Concerning the development and structure of the American *Lycopodium* gametophytes, the writer hopes to deal in a later paper.

MARQUETTE, MICH.

A COMPARATIVE STUDY OF WINTER AND SUMMER LEAVES OF VARIOUS HERBS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 224

J. P. STOBER

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A COMPARATIVE STUDY OF WINTER AND SUMMER LEAVES OF VARIOUS HERBS

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J. P. STOBER

Introduction

The structure of most plants varies with the habitat and even with the varying conditions of the same habitat. This has been emphasized by GREVILLIUS,[1] CHERMEZON,[2] COWLES,[3] STARR,[4] and others. GREVILLIUS made an extensive comparative study of vegetation growing on the island Öland. He compared the plants of a dry, rocky, treeless plain (alvar) with the same species growing in favorable regions. The former he calls alvar forms; the latter, normal forms. The alvar forms, in general, were more hairy and had a more highly cutinized and thicker epidermal wall, a more compact palisade parenchyma, and more closely crowded stomata than the normal forms.

These structural peculiarities due to environmental changes may be observed readily in almost any plastic plant. *Oenothera*

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[1] GREVILLIUS, A. Y., Morphologisch-anatomische Studien über die xerophile Phanerogamenvegetation der Insel Öland. Bot. Jahrb. **23**:24–108. 1897.

[2] CHERMEZON, H., Recherches anatomiques sur les plantes littorales. Ann. Sci. Nat. Bot. **12**:117–313. 1910.

[3] COWLES, H. C., The ecological relation of the vegetation of the sand dunes of Lake Michigan. BOT. GAZ. **27**:95–117, 167–202, 281–308, 361–391. 1899.

[4] STARR, ANNA M., Comparative anatomy of dune plants. BOT. GAZ. **54**: 265–305. 1912.

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biennis, for example, when growing in a dry, sterile soil and exposed to strong wind and maximum sunlight, is found to have smaller and thicker leaves, more perfectly developed palisade parenchyma, a more hairy and a more densely cutinized epidermis, and, in general, a more xerophytic structure than the same species growing under more favorable conditions.

A similar structural difference is apparent in summer and winter leaves, or in stem and rosette leaves. Winter leaves, as the name implies, exist during the winter, which, in our latitude, is the most unfavorable season of the year. During the winter transpiration becomes relatively excessive because of the reduced rate of absorption, and plants are thus put to the severest test. Sometimes for days at a time the ground is frozen and absorption is practically zero; while during the warmest part of the day considerable transpiration may take place. The plant is thus exposed to the danger of desiccation. Moreover, during the night the most exposed leaves may freeze hard. Toward noon of the following day they may thaw out, presenting a wilted condition as if killed by scalding. However, it is surprising how quickly such leaves will revive as conditions again become more favorable. No sooner is the absorption of soil water resumed than the leaves once more become turgid and resume their wonted appearance, apparently none the worse for the ordeal through which they have passed.

Since winter leaves are exposed to such severe conditions, it would be natural to suppose that they must be quite xerophytic in structure. While this is true to a certain extent, in some respects they are less exposed to unfavorable conditions than stem leaves. This is especially true of winter leaves occurring in rosettes. In rosettes the internodes are extremely short and the leaves thus become closely crowded and overlapping. Since epinasty prevails during the winter, these overlapping leaves lie almost flat on the ground, thus affording maximum protection for each other from sudden changes of temperature, as well as from high winds and excessive transpiration. It is seldom that winter leaves die as the direct result of freezing, and when it is borne in mind that such leaves have a low water content and a high osmotic pressure, thus insuring easier absorption of soil water, the protection would seem ample, regardless of any special protective structures. The stem leaves, on the other hand, are usually borne some distance above the ground and exposed to greater intensity of light, stronger winds, and greater extremes in temperature, humidity, and transpiration.

Method of study

Most of the plants used in this comparative study were collected in the region about Chicago; the remainder, in eastern Pennsylvania. In order to simplify matters, all winter leaves, whether produced in typical rosettes, on prostrate runners, or on basal shoots, will be designated as rosette leaves, and summer leaves will be designated as cauline or stem leaves.

Leaves for study were killed, fixed, and preserved in a 4 per cent solution of formaldehyde in 50 per cent alcohol. Delafield's haematoxylin was used as a general staining reagent. Sections were also treated with chloriodide of zinc, the cellulose wall turning blue, while the cuticle and cutinized portions of the epidermal wall turned yellow. Alcannin tincture imparts a pink color to cutin, but is much slower in its action than chloriodide of zinc.

Unless otherwise specified, all observations were made on the middle of the leaf, from the midrib to the margin. All observations and measurements were made with $\frac{2}{3}$, $\frac{1}{6}$, and $\frac{1}{12}$ in. (oil immersion) objectives, and with a 1 in. micrometer eyepiece with divisions of 0.1 mm. Camera lucida drawings were made of portions of the epidermis for measurement and comparison of epidermal cells and stomata. Chloral hydrate was used as a clearing agent for leaves to facilitate the study of air spaces and packing of mesophyll tissues. Most measurements and counts represent an average of 5-20, depending upon the degree of variability of the objects measured. Measurements are expressed in microns, and counts represent the number in the field under low or high power, which is indicated in each case. Different plants of the same species (in cases where the plants could readily be secured) were studied at different times and the results compared. These results varied only slightly when the plants came from the same habitat, but usually differed considerably in plants from different habitats.

With such a tendency to variation in plants, few measurements and counts can be regarded as absolutely fixed, but the final results in any case do not materially affect the principles involved.

Epidermal hairs

There is considerable variation in the kind, number, size, and distribution of epidermal hairs, not only in different plants of the same species, but also on different leaves of the same plant, or even on different parts of the same leaf. Some plants, such as *Oenothera biennis*, vary greatly when grown under different physical conditions. In a low, moist, and comparatively shady habitat the leaves of *Oenothera* are thin, and the hairs rather weak and comparatively few and scattered. On a dry slope or bank along the roadside, the leaves are decidedly thicker, and the hairs stouter and very much more abundant; while under intermediate conditions corresponding variations have been observed.

Oenothera is an extremely plastic plant, responding readily to changed conditions of environment. *Leonurus Cardiaca*, *Lepidium virginicum*, *Capsella Bursa-pastoris*, and others also show some variations, but not to the same extent as *Oenothera*. *Verbascum Blattaria*, on the other hand, is glabrous no matter under what physical conditions it may be growing. Occasionally, when growing on a dry bank along a dusty roadside, a few hairs may be found on the ventral side of the midrib of the lower stem leaves and upper rosette leaves. This plant is extremely rigid and does not at all, or but slightly, yield to changing conditions of environment. It is perhaps a good illustration of a congenital mesophyte.

In studying the number and distribution of hairs, *Oenothera biennis*, *O. rhombipetala*, *Leonurus Cardiaca*, *Lepidium virginicum*, *Capsella Bursa-pastoris*, and *Hieracium paniculatum* were selected as types. Care was taken to collect both the stem and rosette plant of each species in the same or as nearly the same habitat as possible. Five plants of each species were studied, and the counts for each particular kind of hairs were averaged and tabulated. The field of the low power of the microscope was adopted as the unit area of observation, and the average of 5 or more counts was taken as the number for each area under observation.

From the tabulated results of observations made on these species of plants and a careful study of a number of other species, the following conclusions can be formulated. (1) Epidermal hairs are most abundant on the upper stem leaves, and decrease, as a rule, to the lowest stem leaves, and from the upper to the lowest rosette leaves. On the basal leaves of both stem and rosette are found the smallest number of hairs. (2) Hairs are also more abundant on the lower than on the upper surface of the leaf, usually being most abundant on the ribs, veins, and margin of the leaf. (3) Hairs are most abundant toward the base of leaves, although in basal stem and rosette leaves the reverse is usually the case. (4) Young leaves are more hairy than older ones. This may be due partly to the fact that in young immature leaves the epidermal cells have not yet reached their mature size and therefore the hairs will of necessity be more crowded than in a mature leaf. This diminished hairiness in older leaves also may be due in part to the fact that hairs in the course of time may break off, or for some reason or other drop off, and thus reduce the number per unit area of surface. (5) Exposure to sun, wind, and other desiccating influences tends to increase the hairiness in the upper stem leaves. Transpiration, wind, moisture, and character of soil are undoubtedly potent factors in determining hair production, but that these are not the only factors is clearly shown by the fact that young leaves just emerging from buds, and therefore most protected, are usually most hairy, sometimes even tomentose.

As stated before, some leaves are most hairy toward the base, where the leaf is most protected from those influences that would ordinarily tend to produce hairiness. It is difficult also to see why *Verbascum Thapsus* and *V. Blattaria* should grow side by side, the one glabrous and the other extremely hairy. So far as hairiness is concerned, it would seem that the former is a congenital xerophyte while the latter is a congenital mesophyte.

It is difficult also to see that hairiness is beneficial to plants, and that these epidermal outgrowths protect the plant against excessive transpiration, against the ravages of animals and parasites of various kinds, against excessive sunlight, etc., when *Verbascum Blattaria*, entirely devoid of hairs and with only a slightly thicker epidermal wall, is fully as successful in the struggle for existence as *V. Thapsus* growing by its side, so thoroughly protected by an abundance of epidermal hairs. It is not difficult to see that the woolly coating may be advantageous to young leaves, just emerging from the bud; but it is extremely difficult to find any advantage in the few simple and stellate hairs scattered over the leaves of *Lepidium* and *Capsella*.

Stomata

In over two-thirds of all the plants studied the stomata were found to be more abundant on stem than on rosette leaves. Sometimes this difference in number is only slight, but sometimes, as in *Mitella diphylla*, *Lepidium virginicum*, *Monarda punctata*, *Aquilegia canadensis*, *Campanula rotundifolia*, *Capsella Bursa-pastoris*, and *Geum album*, this difference is considerable. Stomata are also most abundant on the lower side of the leaf. This is true of about 80 per cent of all the plants studied. This difference is most pronounced in leaves that have their upper and lower sides well developed, such as the broad mesophytic rosette leaves. Narrow, xerophytic stem leaves, such as have both sides almost equally exposed to light and air, have approximately the same number of stomata on both sides. The more xerophytic the leaves, the greater are the number of stomata as compared with the corresponding mesophytic leaves. As a rule, the size of stomata is correlated with the number. The larger the number of stomata on a given leaf surface the smaller they are. This was found to be true in over 60 per cent of the specimens compared. Broad mesophytic rosette leaves have fewer but larger stomata on a given surface than the corresponding narrower, more xerophytic stem leaves. In these there is a larger number of stomata per unit surface, but the stomata are decidedly smaller in size.

There also seems to be a correlation between the number and size of stomata, and the size of epidermal cells. The broad rosette leaves have, as a rule, larger epidermal cells. With these larger cells are associated fewer but larger stomata.

Anterior-posterior orientation of stomata is noticeable in the stem leaves of *Campanula rotundifolia*, *Linaria canadensis*, *Arabis* *lyrata*, *A. brachycarpa*, *A. laevigata*, and *Satureja glabra;* and in both stem and rosette leaves of *Artemisia caudata* and *Lechea villosa*. All these leaves are linear or oblong. Not all linear or oblong leaves have their stomata longitudinally oriented, but such orientation is characteristic of linear and oblong leaves, especially if the epidermal cells are longitudinally elongated.

The stomata of the species investigated are not sunken below the surface in either stem or rosette leaves, except in the sand dune xerophytes, *Artemisia canadensis* and *A. caudata*, where they are depressed about half the thickness of the epidermis. In a few instances the stomata seemed even to be elevated slightly above the surface. In *Mitella diphylla*, *Leonurus Cardiaca*, *Aquilegia canadensis*, and *Chelidonium majus*, the stomata are confined to the ventral surface of the leaf.

Rosette stomata are not only larger but also more elongated than stem stomata. Stem stomata are not only smaller but also more nearly round than rosette stomata. Perhaps the number of stomata ought to be correlated with the mass of the chlorenchyma. The smaller number of stomata in the broad, thin (frequently thicker than stem leaves), mesophytic rosette leaves, when compared with the smaller mass of chlorenchyma to be aerated, may be relatively as abundant as the larger number per unit surface in the long, narrow, thick xerophytic stem leaves, where a larger mass of chlorenchyma must be aerated through a given surface area; that is, the number of stomata is correlated with the amount of chlorenchyma to be aerated, and not with the mere surface area of the leaf. The number of stomata also seems to be correlated with the thickness of the cuticle and cutinized outer wall of the epidermis. The greater the thickness, the less is the possibility of gases passing through, and the greater is the need for stomata. It is probably for these two reasons, the greater mass of chlorenchyma per leaf surface and the greater thickness of the cuticle and cutinized outer wall of the epidermal cells, that xerophytic leaves have an increased number of stomata in a given surface area. The relatively thinner and frequently more shaded rosette leaves are broader and have a thinner cuticle, a thinner outer epidermal wall, and a greater development of air lacunae. Such leaves need fewer and are provided with a smaller number of stomata. Stomata are not needed for transpiration, since transpiration is believed to be a necessary evil. It seems strange, therefore, that in xerophytic leaves, where there is effected the greatest protection against the loss of water by the development of a thick cuticle and a thick outer epidermal wall, there should be the development of a large number of stomata, thus facilitating the loss of water from the plant through stomatal transpiration.

In leaves whose outer epidermal wall and cuticle are thin, there is less need of stomata to facilitate exchange of gases in photosynthesis and respiration, since under such circumstances considerable interchange of gases can take place through the epidermis. There is no doubt that mesophytic rosette leaves with a reduced number of stomata have an ample supply of stomata to meet their needs. Moreover, rosette leaves are close to the soil and are therefore more advantageously situated for the intake of carbon dioxide than are stem leaves. In stem leaves the pressure of CO_2 cannot accumulate beyond 0.0003 A, or about 0.22 mm. Hg, since above this pressure it diffuses outward. But in rosette leaves close to the ground, where the exhalation of CO_2 from the soil often increases the CO_2 to 10 or more times the normal amount, a much higher pressure of CO_2 may accumulate. This increased amount of CO_2 in rosette leaves is available for carbohydrate synthesis in all cases where the leaves are not too much shaded. But since plants under normal conditions receive much more energy of sunlight (about 4 or 5 times as much) than is necessary to synthesize the small amount of available CO_2, rosette leaves are most advantageously situated for photosynthesis in spite of the reduced number of stomata and the diminished amount of light. These facts have an important bearing upon the development of chlorenchyma and air spaces in rosette leaves.

Epidermal cells

In monocotyledonous plants the epidermal cells are usually elongated. In dicotyledonous plants they are generally elongated along the ribs and larger veins, but elsewhere they may be polygonal and nearly isodiametric in outline, or entirely irregular. The shape of the leaf, to a certain extent, determines the shape of the epidermal cells. In narrow and elongated, or linear, leaves, such as those of the stems of *Arabis brachycarpa*, *A. lyrata*, *Linaria villosa*, and *Artemisia caudata*, the epidermal cells also are elongated or linear. In such elongated or linear cells the lateral walls are quite regular. The upper epidermal cells, however, are usually more regular than the lower, except in such stem or rosette leaves as are almost equally exposed to light. Such leaves have both surfaces almost equally exposed to desiccating influences, hence the shape and size of the epidermal cells on both sides of the leaf are practically the same. This is very apparent in such xerophytic stem leaves as those of *Linaria villosa*, *Arabis lyrata*, *A. brachycarpa*, *A. laevigata*, and *Campanula rotundifolia*.

The shape and size of epidermal cells vary greatly, not only in different species and in individuals of the same species, but even in stem and rosette leaves of the same individual. There may even occur considerable variation in different parts of the same leaf. Thus in *Leonurus Cardiaca* the sinuosity of the lateral walls increases slightly from the lower to the upper stem leaves. In *Geum album* the sinuosity seems to increase from the upper to the lower rosette leaves. In *Lepidium virginicum* the sinuosity is practically the same from the upper stem leaves to the lowest rosette leaves. However, the sinuosity in the lower epidermis, in the case of *Lepidium*, is greater than in the upper epidermis. The lateral walls of the lower epidermal cells are, as a rule, more sinuous than those of the upper epidermis, and in the majority of instances (70 per cent) the sinuosity is greater in rosette than in stem leaves. Sinuosity of the lateral wall culminates under the most mesophytic conditions. Increased transpiration tends to produce relatively straight lateral walls. Hence we find the epidermal walls of the stem leaves less sinuous than those of rosette leaves, and those of the upper surface of both stem and rosette leaves less sinuous than those of the lower surface, since stem leaves are more xerophytic than rosette leaves, and the upper side of leaves more xerophytic than the lower side. Sinuosity of lateral epidermal walls is not known to be of special significance to plants. It may add a little to the strength of the epidermis and afford a larger diffusion surface for substances passing from cell to cell. No chloroplasts are present in epidermal cells except in guard cells, and, to a slight extent, in winter leaves of *Leonurus Cardiaca*.

As to size, the upper epidermal cells are larger than the lower, and, with few exceptions, the epidermal cells of rosette leaves are larger than those of stem leaves. In 80 per cent of all observations made the epidermal cells of rosette leaves were found to be larger than those of stem leaves. The size of epidermal cells is somewhat correlated with the size of leaves, the larger leaves having the larger epidermal cells; but there are so many exceptions to this that such a general statement is not warranted.

The vertical diameter of epidermal cells is greater, as a rule, in rosette than in stem leaves (true of 80 per cent of cases), in the upper than in the lower epidermis, and usually increases from the apex toward the base of the leaf. In the middle rosette leaves the maximum diameter is usually found in the middle of the leaf. In *Capsella* there is a gradual increase from the upper stem to the lowest rosette leaves. As a rule, the maximum diameter is attained in both the middle stem and rosette leaves.

Blade, epidermal wall, and cuticle

The blade decreases in thickness from the apex to the base of the leaf. It also decreases from the upper to the basal leaves. This is less apparent in middle leaves, where the leaf sometimes increases in thickness from apex to base, or where the maximum thickness of the blade occurs in the middle of the leaf. Those leaves or parts of leaves most shaded are usually thinnest. Rosette leaves are thicker than stem leaves, owing to a greater development of spongy parenchyma. This is true more particularly of the middle and basal stem and rosette leaves. The upper stem leaves, especially the apical portions of those leaves, are frequently thicker than the corresponding portions of rosette leaves. The blade, in most instances, also appears thicker than the blades of stem leaves. Notable exceptions are *Arabis lyrata*, *A. laevigata*, *Linaria canadensis*, *Leonurus Cardiaca*, *Campanula rotundifolia*, and *Monarda punctata*. All these species, except *Leonurus Cardiaca*, have either linear or oblong lanceolate stem leaves, while the basal leaves are broad and thin. These are plants that have almost a typical xerophytic shoot and a mesophytic rosette.

The outer epidermal wall is decidedly thicker in stem than in rosette leaves. In each of the 3 types considered, *Lepidium virginicum*, *Capsella Bursa-pastoris*, and *Chrysanthemum Leucanthemum*, the stem leaves are borne considerably above the ground and rather widely separated from each other, thus exposing them freely to air, sunlight, and desiccating winds. The rosette leaves, on the other hand, are close to the ground and considerably shaded; hence we should naturally expect this difference in thickness of epidermal cell walls. There is a slight tendency for the wall to diminish in thickness from the apex to the base of the leaf. The maximum thickness is usually reached in middle stem and apical rosette leaves, while the maximum thinness is probably to be found in the lowest rosette leaves. The outer epidermal wall on the upper surface of the stem leaves is but slightly thicker than that of the lower, especially in those upper stem leaves that grow obliquely upward so as to expose both surfaces almost equally. In the lower stem and rosette leaves this difference is much greater, the epidermal wall on the lower side being considerably thinner. The thickness of the cuticle varies with the thickness of the outer epidermal wall, the thickest wall having the thickest cuticle. The cuticle of the stem leaves of the types treated is decidedly thicker than that in the rosette leaves. It is thicker on the upper than on the lower surface of the leaf, except in the upper stem leaves, where both surfaces are about equally exposed. Here the lower cuticle is almost as well developed as the upper. The greatest decrease in thickness of cuticle is observable in the basal rosette leaves.

In interpreting the facts set forth it must be borne in mind that only middle stem leaves are compared with middle rosette leaves, and that whatever conclusions may be deduced must rest upon this comparison. Most plants have their rosettes better protected than their shoots. In 83 per cent of 30 plants observed, the cuticle is thicker in rosette than in stem leaves. In at least 75 per cent of the number the epidermal wall is also thicker in rosette than in stem leaves. Thus it seems that when the effective means of protection of the middle stem leaves and the middle rosette leaves are compared, the preponderance of protection is in favor of the rosette leaves. However, it must be borne in mind that the difference in thickness of wall and cuticle in a number of instances is so slight as to be almost negligible. Moreover, in notable instances the stem leaves have a decidedly thicker wall and cuticle. This is true of *Chrysanthemum Leucanthemum*, *Capsella Bursa-pastoris*, *Artemisia caudata*, *Satureja glabra*, *Scutellaria parvula*, and others. *Chrysanthemum* has broad, spatulate rosette leaves on long slender petioles, while the stem leaves are oblong or oblanceolate, and have a decidedly xerophytic form and structure. The stem leaves of *Lepidium*, *Capsella*, *Satureja*, and, to a certain extent, *Scutellaria*, in like manner have a decidedly xerophytic form and structure as compared with their corresponding rosette leaves. *Artemisia* is one of those sand dune xerophytes whose stem and rosette leaves are finely dissected and almost equally exposed, and hence almost equally xerophytic in form and structure. In such mesoxerophytes as *Verbascum Thapsus*, whose leaves are thoroughly protected by a woolly coat of branching multicellular hairs, the difference in protection of stem and rosette leaves is also slight. Some plants, therefore, seem to have xerophytic shoots and mesophytic rosettes; others show a tendency to xerophytic rosettes and mesophytic shoots; while in still others the distinction is not evident.

Chlorenchyma

The apical, middle, and basal stem and rosette leaves of certain plants were studied with a view to determining the similarities and differences of the chlorenchyma of the corresponding regions of the stem and rosette leaves of the same plant. For example, an apical stem leaf and an apical rosette leaf would be selected for comparative study. Sections through the apical region of the stem leaf were then studied and the results compared with those obtained from a similar study of corresponding sections of the rosette leaf. Sections through the middle and basal regions of the leaves were similarly studied and compared. After the apical leaves were thus studied. the middle stem and middle rosette leaves, as well as the basal stem and basal rosette leaves, were similarly studied. The thickness of the leaf, the thickness of the palisade parenchyma, the thickness of the spongy parenchyma, the number of the palisade layers of cells, and the average size of the cells of each layer, together with the size, shape, and arrangement of the cells of the spongy parenchyma, were the leading points of observation in this comparative study. All measurements are expressed in microns, and were made approximately 800 μ from the midrib of the leaf. For want of space the tabulated results cannot be given; a general summary in each case must suffice.

LEPIDIUM VIRGINICUM.—In the upper stem leaves the palisade parenchyma is almost equally developed in both the upper and lower side of the leaf. This may be due to the fact that the leaves stand at a very acute angle to the stem and are almost equally illuminated. In the middle stem leaves a lower palisade tissue is found only in the apical region of the leaf. No lower palisade layers are found in the lower stem leaves or in any of the rosette leaves. The palisade layers of the upper stem leaves are quite compact. The cells reach a maximum length in the middle stem leaves. In the basal stem leaves the cells become larger and more rounded, the layers are less closely packed and less definitely organized. The palisade cells of the rosette leaves are larger, having a decidedly greater diameter, and on the whole are less compactly arranged than in stem leaves. The upper and middle stem leaves have the thickest outer epidermal wall and cuticle. This is also true of the upper rosette leaves when compared with the lower. The difference, however, between the thickness of the epidermal wall and cuticle of the apical and basal leaves is much greater in rosette than in stem leaves.

CAPSELLA BURSA-PASTORIS.—The outer epidermal wall of stem leaves is thicker in the upper than in the basal leaves, attaining a maximum in the middle leaves. The cuticle is proportionally thickest in the upper leaves and thinnest in the proximal part of the basal leaves. Similar conditions obtain in the rosette leaves, except that the contrast between apical and basal cells is less pronounced. Palisade tissue is best developed in both upper stem and upper rosette leaves. Palisade cells are slightly longer and decidedly thicker in rosette than in stem leaves. The cells of the spongy parenchyma are decidedly more irregular in rosette leaves, and the tissue contains a maximum of air spaces. Palisade tissue is least developed in basal stem and rosette leaves, as well as in the basal region of the leaves themselves.

CHRYSANTHEMUM LEUCANTHEMUM.—The outer epidermal wall and cuticle of the upper and middle stem leaves are very much alike in thickness, but both are decidedly thicker than the corresponding epidermal wall and cuticle of the basal stem leaves, the latter being only about one-half as thick. Rosette leaves do not differ much from each other in the thickness of epidermal wall and cuticle, but the maximum thickness may probably be found in the middle leaf. Rosette leaves, as a whole, have a thinner epidermal wall and cuticle than stem leaves, being only one-half to two-thirds as thick. The palisade tissue is better developed, as a whole, in stem than in rosette leaves, and decidedly better developed in both stem and rosette leaves in apical and middle leaves than in basal leaves. The spongy parenchyma is slightly better developed in rosette leaves and in both kinds of basal leaves. Here is found also the maximum development of air spaces.

OENOTHERA BIENNIS.—The stem leaves are thickest in the apical region and gradually become thinner toward the base. There is also a gradual increase in thickness from the apical to the basal stem leaves. The upper rosette leaves are thickest in the apical region and become thin toward the base of the leaf. In the middle and lower rosette leaves the greatest thickness is found in the middle region. From this region they gradually become thinner toward both the apex and base of the leaf.

The spongy parenchyma in stem leaves gradually diminishes from the apical to the basal region of the leaf, but there is a gradual increase in amount from the apical to the basal leaves. In the upper rosette leaves the spongy parenchyma also gradually decreases from the apex of the leaf to the base. In the middle and lower rosette leaves, however, the greatest percentage of spongy tissue is found in the apical and basal regions. In stem leaves the palisade tissue is most developed in the apical region and least in the basal region. The maximum development is probably found in the apical and middle regions of the basal leaves. The palisade cells of rosette leaves are decidedly broader or thicker than those in stem leaves, but are relatively slightly longer. The maximum development is found in the apical and middle regions of the leaf, or in those parts of the leaves having the greatest exposure to light and other desiccating influences.

The largest epidermal cells are found in the middle region of both stem and rosette leaves. It is also in the middle of leaves that both upper and lower epidermal cells have the greatest vertical diameter. The outer epidermal wall and cuticle of stem leaves are thickest in the apical leaves, and gradually become thinner toward the basal leaves. In the upper stem leaves the outer wall and cuticle diminish from the apical to the basal region. In the middle and basal stem leaves there is less difference, and in the lowest leaves there is practically no difference in thickness of the epidermal wall and cuticle in different regions. In the upper stem leaves there is not much difference in the thickness of the epidermal wall and cuticle of the upper and lower sides of the leaf; but in the lower stem leaves the thickness is decidedly greater in the upper than in the lower epidermis. The greatest difference in thickness is found in the lowest leaves.

In rosette leaves the situation in thickness of epidermal wall and cuticle is similar to that found in stem leaves. In the upper rosette leaves, however, there is a greater difference in thickness of wall and cuticle between the apical and basal regions of the leaf. The hairs on both stem and rosette leaves are longest and most abundant on the midrib and have larger veins than elsewhere. The hairs are most abundant on the upper stem leaves and gradually diminish in number and size to the basal leaves, where they are quite small (except on veins) and only half or even less than half, as abundant. On the upper leaves they are longer and more abundant on the lower than on the upper surface, and increase in length and abundance from the apex to the base. On the middle stem leaves they are similar in size and abundance on both sides of the leaf, but slightly decrease in number from apex to base. On the basal leaves the hairs are considerably reduced in size, but otherwise the situation is similar to that found in middle stem leaves. On rosette leaves the hairs are slightly more abundant on the upper than on the lower surface, and gradually diminish in size and number toward the basal leaves. On the lower side of the basal leaves there are very few hairs except along the margin, where they are long and abundant. The chlorenchyma contains an abundance of needle-shaped crystals of calcium oxalate, arranged in bundles (raphides). These raphides are slightly more abundant in rosette than in stem leaves.

VERBASCUM BLATTARIA.—In this species the palisade tissue is best developed in the floral leaflets and in the upper stem leaves. Here the layers are well organized and compact, and the cells reach their maximum length. In the basal stem leaves the palisade cells vary considerably in length, some being quite long, while others are quite short. Moreover, the layers are poorly organized. In the middle and upper stem leaves 3 layers are well organized, a fourth layer being only partly organized. In the basal leaves there is no trace of a fourth layer. The thickness, or transverse diameter of the palisade cells, also increases appreciably from the upper to the lowest stem-leaves. In rosette leaves there is a gradual increase in the size of palisade cells from the upper to the basal leaves. In the latter the palisade tissue is poorly developed, the cells being very irregularly and loosely arranged, and scantily supplied with chloroplasts.

With the exception of a few hairs on the ribs of rosette leaves and lowest stem leaves, this plant is devoid of hairs. The basal stem leaves and rosette leaves have the largest epidermal cells, which also have the largest vertical diameter. The upper epidermal cells of both stem and rosette leaves always have a decidedly greater vertical diameter than the cells of the lower epidermis.

The floral leaflets and upper stem leaves have the thickest outer epidermal wall and cuticle. In these leaves there is very little difference between the upper and lower epidermis. In the upper rosette leaves we also find a thicker epidermal wall and cuticle, but the difference is less pronounced than in stem leaves.

A summary of the comparative study of the upper, middle, and lower stem leaves and the corresponding upper, middle, and lower rosette leaves, based upon the 5 species just considered, and in addition *Leonurus Cardiaca* and *Verbascum Thapsus*, is as follows.

In general the lowest stem and rosette leaves, as well as the basal part of all leaves, are most protected and most shaded, and therefore have the most mesophytic structure. The leaves are thinnest; the outer epidermal wall and cuticle are thinnest; the palisade parenchyma is developed most poorly; and spongy parenchyma, containing a maximum of air spaces and a minimum of chloroplasts, is developed most highly.

The upper stem leaves are relatively xerophytic in structure. This is especially true of the apical region of these leaves. We frequently find the maximum thickness of leaf, maximum thickness of epidermal wall and cuticle, and a maximum development of palisade tissue, which in many instances develops almost equally on both sides of the leaf. The middle and lower stem leaves are almost invariably thinner than the corresponding rosette leaves. The spongy parenchyma is better developed in rosette than in stem leaves. This was true of 75 per cent of all sections studied.

The palisade parenchyma in stem leaves is better organized, more compact, and the cells relatively longer and narrower as compared with the thickness of the leaf. In rosette leaves the layers of palisade tissue are frequently less perfectly organized, less compact, and the cells larger. Palisade cells of rosette leaves are decidedly broader and usually longer than those of stem leaves; but the amount of palisade tissue and the length of the cells, when compared with the average thickness of the leaves, are less in rosette than in stem leaves. The absolute length of palisade cells in the first layer is greater in rosette leaves than in corresponding stem leaves in 70 per cent, in the second layer in 55 per cent, and in the third layer in 28 per cent of all sections studied. In 30 per cent of all stem sections studied the second palisade layer was not developed. The same was found to be the case in 29 per cent of rosette sections studied. Likewise, the third palisade layer was not developed in 81 per cent of all stem sections studied, or in 66 per cent of all rosette sections studied. The number of sections considered in each case was the same (75 stem and 75 rosette sections). With the exception of the upper stem leaves, where the upper and lower epidermis frequently have an outer wall of approximately equal thickness. the upper epidermis has a thicker wall than the lower. In 93 per cent of the cases the outer epidermal wall and cuticle of stem leaves were found to be thicker in stem than in rosette leaves. The thickest epidermal walls are usually found in the outer two-thirds of upper stem leaves. On the other hand, rosette leaves have epidermal cells with the largest vertical diameter and contain a maximum of air spaces.

Lepidium virginicum and *Capsella Bursa-pastoris* produce both summer and winter rosettes. When these rosettes are compared, it is found that summer rosettes have slightly thicker leaves (thinner in *Capsella*), a thicker cuticle, and a thicker outer epidermal wall. The palisade parenchyma also is better developed. There are frequently more layers, and the cells are longer and narrower. These differences are most pronounced in *Lepidium*.

SUMMARY ON CHLORENCHYMA.—Typical xerophytic leaves have a relatively compact and well developed palisade tissue; also a relatively small amount of spongy parenchyma with small air spaces. The mechanical tissue is usually also better developed than in mesophytic and shade leaves. Since rosette leaves are usually broad, close to the ground, frequently more or less shaded, and therefore in most respects better protected than stem leaves, it should not be surprising if the former were found to be more mesophytic than the latter. That this seems to be true, at least of the forms studied, is shown by the following deductions.

1. Rosette leaves, as a rule, have a greater amount of chlorenchyma than stem leaves. This is true of at least 80 per cent of all plants studied.

2. Rosette leaves have a greater amount of spongy parenchyma than stem leaves, although the percentage of the chlorenchyma is slightly greater in the latter than in the former.

3. The percentage of air spaces in both palisade and spongy parenchyma is also greater in rosette than in stem leaves. This is true of about 86 per cent of all plants studied. In a considerable number of instances, however, the differences are slight.

4. The number of palisade layers is much the same in both kinds of leaves, but the average length of palisade cells, in at least

80 per cent of the types studied, is greater in rosette than in stem leaves. This is correlated perhaps with the greater thickness of the chlorenchyma in the former. The thickness of palisade cells, in at least 90 per cent of all cases, is also greater in rosette than in stem leaves.

5. The average size of spongy parenchyma cells is also greater in rosette than in stem leaves. This is true of about 90 per cent of all plants studied.

6. Sclerenchyma tissue seems to be about equally well developed in ribs and veins of both kinds of leaves. The conductive tubes in veins of approximately equal size have a slightly larger lumen and a wall slightly thicker in rosette than in stem leaves. The conductive system of rosette leaves is better developed in rosette than in stem leaves, although this rule is not without exceptions.

On the whole, therefore, it may be said that, so far as the structure of chlorenchyma is concerned, stem leaves are more xerophytic in structure than rosette leaves, although the latter appear to be more xerophytic so far as the greater thickness of epidermal wall and cuticle are concerned. In some instances the xerophytic character of stem leaves, as compared with the rosette leaves of the same plant, is so pronounced as to be easily detected with the naked eye.

Conclusions

1. Hairs are most abundant in the upper stem leaves and decrease to the basal leaves; they are also most abundant in the upper rosette leaves and decrease to the basal leaves. In general, however, the stem leaves are more hairy than the rosette leaves.

2. Stomata are usually smaller, more nearly round, and more abundant, per unit area, on stem than on rosette leaves.

3. As a rule, the epidermal cells of rosette leaves are larger than those of stem leaves and have more sinuous lateral walls. The shape of the cells is usually correlated with the shape of the leaf.

4. The blade of rosette leaves is thicker than that of stem leaves, chiefly owing to a greater development of spongy parenchyma. This is not true, however, of stem leaves that are long,

narrow, and of a decidedly xerophytic form and structure as compared with rosette leaves.

5. The outer epidermal wall of rosette leaves is thicker, as a rule, than in stem leaves. The maximum thickness occurs in middle stem and apical rosette leaves. The thickness of the cuticle varies with the thickness of the epidermal wall, the thickest walls having the thickest cuticle. Rosette leaves in the large majority of instances have the thickest cuticle. The preponderance of epidermal protection is in favor of rosette leaves. In stem leaves of xerophytic form the preponderance of epidermal protection is in favor of stem leaves.

6. In a comparison of the different stem and rosette leaves of the same plant it is obvious that the lowest stem and lowest rosette leaves, as well as the basal part of all leaves, have the thinnest epidermal wall, thinnest cuticle, the most poorly developed palisade tissue, the maximum development of spongy tissue and air spaces, and the minimum development of chloroplasts. The upper stem leaves are relatively xerophytic in structure, especially in the apical region of these leaves. The middle and lower stem leaves are usually thinner than the corresponding rosette leaves. The palisade parenchyma in stem leaves usually is better organized, more compact, and the cells relatively longer and narrower, as compared with the thickness of the leaf, than in rosette leaves. The thickness of palisade cells of rosette leaves is greater, in most cases, than in stem leaves. This is also true of the absolute length in the great majority of instances.

7. When the chlorenchyma in middle stem and middle rosette leaves is compared we may conclude: (1) that rosette leaves, in most cases, have a greater amount of chlorenchyma than stem leaves (this is especially true of spongy parenchyma); (2) that in most cases rosette leaves also have more air spaces than stem leaves; (3) that there is little difference in the number of palisade layers in the two kinds of leaves, but in most cases the absolute size of the palisade cells (length and thickness) is greater in rosette than in stem leaves; (4) that the average size of cells of the spongy parenchyma is also greater in rosette than in stem leaves; (5) that sclerenchyma tissue is about equally developed in both kinds of

leaves, but the conductive tissue is slightly better developed in rosette than in stem leaves.

On the whole, typical rosette leaves, where there is considerable shading and protection, are decidedly more mesophytic than stem leaves. In winter leaves on stolons or runners there is a tendency toward greater xerophytism than in stem leaves, but on the whole the rosette leaves are more mesophytic in structure than stem leaves.

In conclusion, I desire to acknowledge my indebtedness to Dr. H. C. Cowles and Dr. J. M. Coulter for helpful suggestions and advice in this work.

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MORPHOLOGY OF KETELEERIA FORTUNEI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 225

(WITH PLATES VII AND VIII AND THREE FIGURES)

A. H. HUTCHINSON

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(WITH PLATES VII AND VIII AND THREE FIGURES)

Since its discovery by Fortune, *Keteleeria* has aroused interest as an endemic Chinese conifer. It was found first near the temple of Foo Chow Foo, and reports of recent explorers locate it near ancient shrines. Whether *Keteleeria*, as seems most probable in the case of *Ginkgo*, was a sacred tree and has been preserved by a religious order is a matter of conjecture.

Because of limited knowledge, even of the gross structure and characteristics, it is not surprising that the form now known as *Keteleeria* has been variously placed by systematists. Lindley named this form *Abies jezoensis*, mistaking it for a Japanese species of that name (14). Murray, in 1862, showed that the form in question differed from *Abies jezoensis* and called it *Abies Fortunei* after the original discoverer. In 1868 Carrière made a new genus *Keteleeria*, naming it after Keteleer, a Belgian horticulturist. Parlatore placed the same form under the genus *Pinus*, as *P. Fortunei;* by Bentham and Hooker it was classified with *Tsuga;* Bentham and Masters again placed it in the genus *Abies;* while by Engler it is described under *Abies*.

Carrière's reasons for making a new genus were that the form in question differs from *Picea*, since it has erect cones; it cannot be included with *Abies* because the cone scales are persistent; and at the same time, in habit and general aspect it resembles *Podocarpus*. Further, Pirotta (14) states that a new genus is justified because of the arrangement of the staminate strobili ("fiori maschili"). The bud of the staminate strobili is borne either in the axils of the leaves of the preceding year or at the apex of a branch. Pirotta regards the cone clusters as true "inflorescences." Each "inflorescence" consists of a short peduncle dilated at the apex into a receptacle-like body which is invested

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by scales; the lower scales are short, those above becoming increasingly longer. The "flowers" are situated on the margin of the dilated peduncle in the form of a circle or false crown, one or two "flowers" being situated near the center of the receptacle. The number of staminate cones in a cluster ranges from 6 to 10. It is rather remarkable that the other genus of Abietineae (*Pseudolarix*) which shows such an arrangement of staminate cones is also an endemic of China. PIROTTA regards this character of sufficient importance to warrant the division of Abietineae into the Euabietineae, including those forms whose staminate strobili are single (*Abies*, *Picea*, *Pseudotsuga*, *Tsuga*), and the Pseudoabietineae, including those forms whose staminate strobili are in clusters (*Keteleeria* and *Pseudolarix*).

PIROTTA (15) has examined also the anatomical structure of the root, stem, and leaves. The root is characterized by a primary axial resin canal, by secondary canals arranged irregularly in the secondary wood, and by the presence of resin-bearing "idioblasts" in the secondary cortex. In the branches there are resin canals and mucilage-bearing "idioblasts" in the primary cortex only. The leaves are bilateral and contain 2 marginal resin canals and also mucilage "idioblasts" in the mesophyll.

The vascular anatomy has been studied also by HOLDEN (11), who says "*Keteleeria* has the wood structure of *Abies*. Ray tracheids are entirely absent even in such primitive structures as the first annular ring, cone-bearing branches, cone axis, and are not recalled after wounding, although there is an abundant formation of traumatic resin canals."

RADAIS (17) has classified conifers according to the distribution of the resin ducts ("caneau sécréteurs") in the megasporophylls. Upon this basis *Keteleeria* is placed with *Cedrus* and *Picea*, cross-sections of the sporophyll, about the middle of the seed, showing resin ducts in both inner and outer parenchyma; in *Tsuga*, *Larix*, *Pseudotsuga*, and *Abies* they are situated in the inner parenchyma; and in *Pinus*. in the outer parenchyma only. This classification, according to the admission of the author, is "surtout artificiel."

The anatomy of the staminate strobilus has been described by AASE (1). The general tendency in the evolution of conifers to proceed from separate bract and scale to the fused bract and scale is noted; in the first group the bract and scale are separate almost to the base of the appendages, and both are about equally prominent. To this group belong *Keteleeria*, *Pseudotsuga*, species of *Abies*, and species of *Larix*.

MALE GAMETOPHYTE.—The multiplication of the cells in the male gametophyte follows the sequence characteristic of the Abietineae. There are 3 successive primary divisions; by the first and second the 2 polar ("prothallial") cells are cut off, the third resulting in the formation of the antheridial cell and the tube nucleus (figs. 1 5). This stage is the most advanced found in available material, and is believed to be the stage at which the pollen is shed. With respect to the development of the male gametophyte at the time of shedding, *Keteleeria* would resemble *Pinus* and might be contrasted with *Abies* and *Picea*. The appendage-like outgrowths of the exine and the inflation of the region between the exine and intine, caused by this growth, result in the production of wings, such as are characteristic of the Abietineae.

The mitoses involved in the development of the male gametophyte are similar to those described for *Abies* and *Picea* (**12,13**). In each of the first 3 mitoses the spindle fibers become oriented in such a way that they surround the polar nucleus; later they radiate from it, appearing in cross-section as tufts of fibers. This peculiarity of the mitotic figure doubtless is associated with the unequal apportionment of the cytoplasm to the resulting nuclei, an inequality which results in more favorable conditions for the more centrally placed nucleus.

In *Keteleeria* the development of the male gametophyte is not uniformly as described. Fig. 5 shows 4 nuclei medianly placed and almost equal in size. The association of such gametophytes with others whose nuclei and cells are unequal and differently placed indicates that the degree of development depends upon conditions, rather than being foreordained. When inclosed by a wall containing little cytoplasm the nucleus soon disintegrates. Fig. 4 shows 3 nuclei which are "prothallial" in nature; the third under ordinary conditions would be regarded as antheridial; in this case the nucleus corresponding in origin to the tube nucleus has taken the central position. In the struggle the nucleus which is most centrally placed gains the ascendency, the others being crowded to the wall.

MORPHOLOGY OF THE OVULATE STROBILUS.—The anatomy of the megasporophyll has been studied by AASE (1). "In *Keteleeria Fortunei* one bundle originates near the base of the gap in the strobilus cylinder and supplies the bract. It remains undivided throughout its course. Two bundles, one from each side of the gap, supply the scale; the two bundles soon unite, forming one inverted bundle, that is, its xylem faces the xylem of bract." However, in the early stages the strands connected with the 2 ovules are separate. The evidence supports the theory that the scale with its megasporangia represents a fertile bud in the axil of the bract.

The material studied shows that at the time the pollen is shed the megasporangium has reached the mother-cell stage. There is only one megaspore mother cell and it is the fourth cell from the epidermis (fig. 9), characters which still further emphasize the relation of *Keteleeria* to the Abietineae.

SIEVE TUBES.—The sieve tubes of *Keteleeria* are large, 8–10× 200–400μ, and are well differentiated. Concerning the sieve tubes of gymnosperms, DEBARY (5) states that "the oblique terminal faces are directed toward the radial planes. Sieve plates are placed in one or two longitudinal rows over the terminal faces and the whole remainder of the radial lateral face. They form roundish spots separated by high intervening portions. These spots are coarsely latticed, while in the cavities of the coarse lattice the very delicate sieve structure is seen." The appearance as seen in a surface view only has been described, and this has led to an erroneous idea of the structure. The description given by HABERLANDT (6) and DEBARY might apply to the structures as represented in fig. 6. However, transverse and tangential sections show no thickenings or depressions in the walls of the sieve tubes. The appearance of "delicate sieve structures" described for radial sections is caused by the presence of granular inclusions in protoplasmic aggregations which are situated on either side of groups of perforations in the sieve tube walls. The protoplasmic masses are connected by delicate strands which penetrate these perforations (figs. 7, 8).

The general tendency in the modification of sieve tubes from the lower to the higher vascular plants is toward an increasingly smaller number of sieve plates. First, there is a decrease in the number of walls upon which the plates occur. In some ferns each sieve tube wall contiguous with the wall of a similar cell bears sieve plates, while in most angiosperms they occur on the terminal walls only. Again, there is a tendency toward diminution in the number of plates on a given surface. In ferns (**5**, p. 180) there are several rows of plates, or they may be closely crowded together; in *Vitis* there are a number of elongated plates on the oblique septae; in cucurbits there is a single plate. In *Keteleeria* the occurrence is limited to the walls seen in radial sections and the oblique terminal walls. This is true of gymnosperms in so far as the records are available. The plates are arranged in a single interrupted series of groups. In this respect *Keteleeria* is much more advanced than *Encephalartos* (**5**, p. 181, *fig. 78*); the latter has plate groups closely distributed over the radial faces. Moreover, the plate groups are much less numerous on the radial faces in *Keteleeria* than on the oblique terminal faces. This is a further advance toward the condition in angiosperms. It seems probable that the investigation of other forms in this respect would give valuable evidence with reference to genetic relationships.

EMBRYO.—The embryo of *Keteleeria* is of considerable morphological interest; it throws light upon the polycotyledonous embryo of Coniferales, and also the meristem of the primary root shows characters heretofore unknown among gymnosperms (**2, 7, 8, 9, 10**). PIROTTA (**16**) has described the seedling.

In the embryo, as found in the mature seed, the following regions occur: cotyledons, leaf bud, and primary root. Beginning at the exterior, a cross-section of the root, taken near the central region (figs. 24, 25), shows the coleorhiza, the cortex, the region of meristematic cells and mucilage cells, and the central axis.

There is a cotyledonary tube which extends throughout approximately two-thirds of the length of the embryo. The cotyledons proper are terminal upon the cotyledonary tube, and their length is about one-sixth of that of the tube. At the base the tube is in the form of a hollow cylinder (fig. 19) above; the inner surfaces become rectangular, then star-shaped in outline (figs. 16, 17); finally, the 4 cotyledonary tips become separate (fig. 16).

Occasional seedlings of *Abies* and *Pinus* have been described (**7, 8, 9, 10**) having limited cotyledonary tubes, but no such pronounced structure as occurs in *Keteleeria* has been recorded. Although the material did not show the earlier stages, it seems evident that the situation here is similar to that existing in angiosperms (**3, 4**); that cotyledons, whether several as in Coniferales, two as in dicotyledons, or one as in monocotyledons, are all similar in origin; that the cotyledonary growth is, primarily, that which results from a meristematic ring about the leaf bud, the number of cotyledons being dependent upon the number of the loci of increased growth. In *Keteleeria* the major part of cotyledonary elongation is uniform throughout the entire ring of the growth region.

With the exception of the central axis, the regions of the primary root are similar to those of other conifers; in *Keteleeria* this region is continuous throughout, while in other conifers described it is broken by the meristematic region. It is evident that such a modification in structure is due to the nature of the meristem. Since the meristem of certain conifers, including *Keteleeria*, is being described in another paper, details may be omitted here.

The differentiation of tissues as they occur in the embryo of the mature seed is advanced beyond the stage usual for conifers (**7, 8, 9, 10**). In the primary root the first cells to become differentiated are those which later become mucilage tubes (shown in black, figs. 15–27). The cells cease to divide and become vacuolate (fig. 11); the nucleus disintegrates; the cells are greatly elongated by division and growth of the surrounding cells and become mucilaginous in content. Similar cells, except that they are much shorter, are formed in the coleorhiza. The cells of the cortex become filled with food materials, generally in the form of starch (fig. 12). The cells forming a hollow meristematic cylinder about the central axis divide in either of two planes (fig. 13). The first xylem elements, having the usual spiral thickenings of the protoxylem, become differentiated in the cotyledons; there are 4 groups of protoxylem, with 2–4 strands in each group, extending

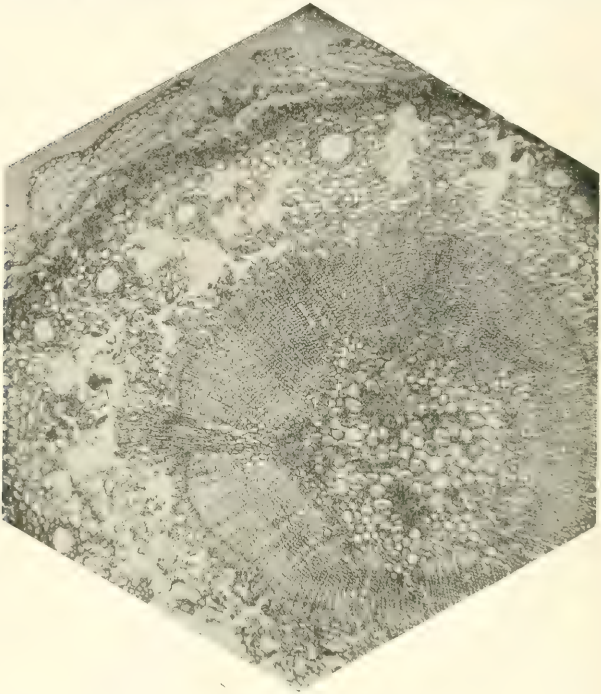

FIG. 1.—Transverse section of a branch showing epidermis, cork cambium, cortex with resin ducts, phloem, xylem, pith, and a leaf trace.

from near the tip of the cotyledon to the base of the cotyledonary tube. The protoxylem is endarch (figs. 16–20). It is significant that at this time there is no xylem present in the primary root or in the leaf bud (stem tip).

The 4 meristematic regions of the cotyledonary tube, with which the protoxylem is associated, and also the meristem of the leaf bud, connect with the hollow meristematic cylinder of the primary root. The central axis extends beyond this junction point, thereby modifying the structure generally known as the cotyledonary plate (fig. 15) (**7, 8, 9, 10**).

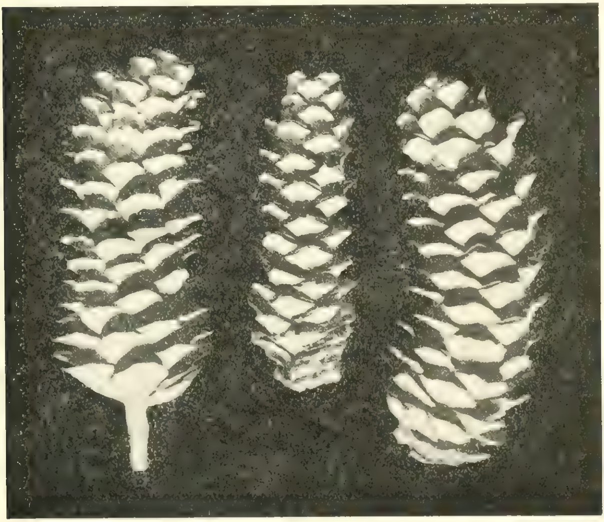

FIG. 2.—Mature ovulate cones

Summary

The following characters of the form in question warrant a genus *Keteleeria*, belonging to the Abietineae.

1. *Ovulate strobilus*.—(1) The cones are erect (text fig. 2); (2) the scales are persistent (text fig. 2); (3) the scale and bract are separate nearly to their bases (text fig. 3); (4) there is a single megaspore mother-cell (fig. 9).

2. *Staminate strobilus.*—(1) The staminate cones are borne in clusters on a fertile branch (text fig. 3); (2) there are 2 abaxial microsporangia on each sporophyll; (3) the pollen is winged (figs. 1–5).

3. *Male gametophyte.*—The pollen is shed in the 4-celled stage, consisting of 2 polar cells, the antheridial cell, and the tube nucleus (figs. 2–3).

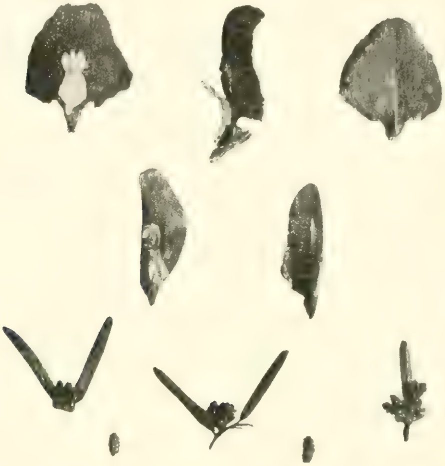

Fig. 3.—Above, megasporophylls with scale and bract separate nearly to the base; below, fertile branches bearing groups of staminate strobili; between, 2 winged seeds.

4. *Vascular anatomy.*—(1) Resin ducts do not occur in the secondary wood except as traumatic responses (text fig. 1) (11); (2) there are no ray tracheids nor are they "recalled by wounding" (11).

5. *Embryo.*—(1) There is an extensive cotyledonary tube (figs. 15–20); (2) a central axis extends throughout the primary root (fig. 15).

6. *Leaves.*—(1) The leaves are spirally arranged on ordinary branches; (2) there are 2 very closely associated vascular strands and 2 marginal resin ducts (**15**).

7. *Sieve tubes.*—These present single interrupted rows of plate groups on the radial and oblique terminal faces. Paired protoplasmic accumulations, one on either side of each plate, are connected by strands which penetrate small perforations in the intervening walls (figs. 6–8). The sieve tubes are more numerous on the oblique terminal faces, an advance toward the angiosperm condition.

8. *Cotyledonary tube.* This is significant in connection with the theories of the origin of polycotyledony.

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EXPLANATION OF PLATES VII AND VIII

FIGS. 1–5.—Male gametophyte; ×1630.

FIG. 1.—First polar cell and second primary mitosis.

FIGS. 2, 3.—Two polar cells, antheridial cell, and tube nucleus; in fig. 3 the wall which later cuts off the antheridial cell has not yet been formed; the radiating fibers may be noted.

FIG. 4.—Three nuclei have been crowded to the wall ("prothallial cells"); tube nucleus is central.

FIG. 5.—Four medianly placed nuclei, alike in size and structure.

FIGS. 6–8.—Sieve tubes with sieve plates; ×1630.

FIG. 6.—From a longitudinal radial section.

FIG. 7.—From a longitudinal tangential section.

FIG. 8.—From a transverse section, a cell from the pith ray being shown also.

FIG. 9.—Megasporangium showing megaspore mother-cell.

FIGS. 10–14.—Cells from different regions of the embryo.

FIG. 10.—Cells from meristem of cotyledon.

FIG. 11.—Cells becoming differentiated to form mucilage tubes.

FIG. 12.—A cortical cell.

FIG. 13.—Cells from meristem of primary root.

FIG. 14.—From protoxylem of cotyledonary tube.

FIGS. 15–27.—Semidiagrammatic drawings of the embryo; ×40.

FIG. 15.—Longitudinal median section; attached numbers indicate region from which accompanying transverse sections have been taken.

FIGS. 16–27.—Transverse sections.

FIG. 16.—Cotyledons.

FIGS. 17–20.—Cotyledonary tube, showing meristematic region and protoxylem (black).

FIG. 20.—Cotyledonary tube and leaf bud.

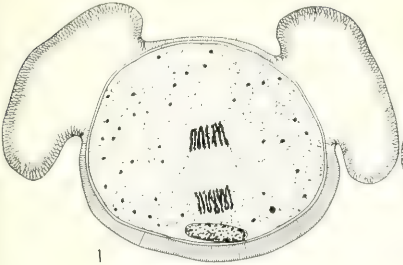
FIG. 21.—Junction of hollow meristematic cylinder of primary root and 4 meristematic regions of cotyledonary tube.

FIGS. 22, 23.—Cortex, region of meristem, and central axis.

FIGS. 24, 25.—Showing 4 regions; coleorhiza, cortex, region of meristem, and mucilage tubes; also central axis.

FIG. 26.—Section of coleorhiza, cortex, and central axis.

FIG. 27.—Section of coleorhiza and central axis.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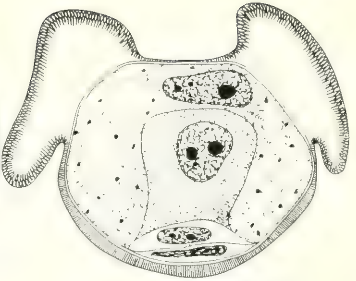
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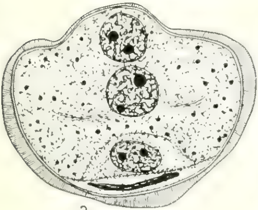
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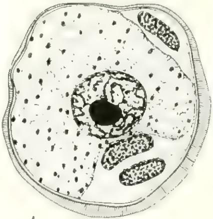
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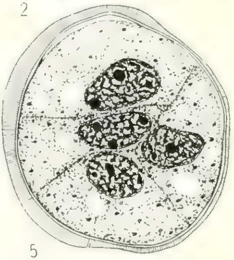
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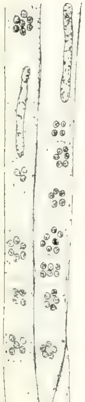
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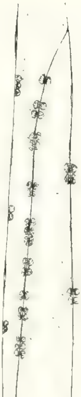
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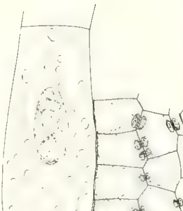
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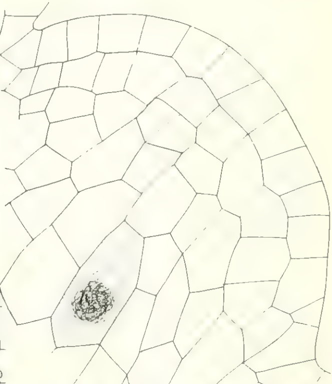
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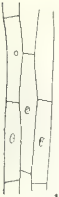

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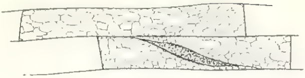

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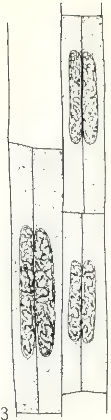

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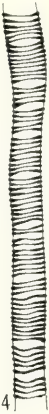

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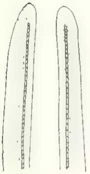

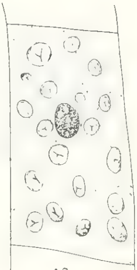

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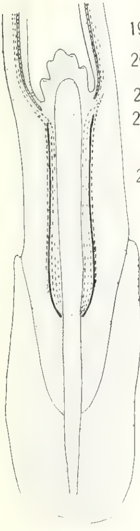

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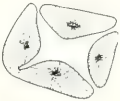

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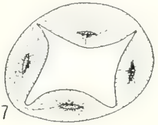

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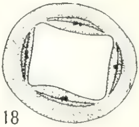

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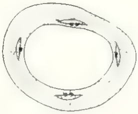

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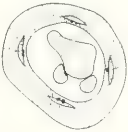

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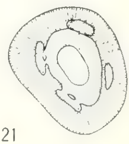

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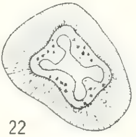

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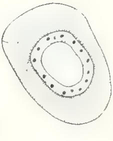

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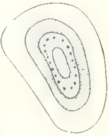

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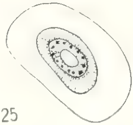

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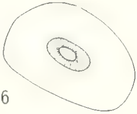

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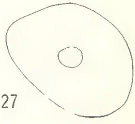

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